ISSN 0388-841 X

BULLETIN
OF THE
NANSEI REGIONAL FISHERIES RESEARCH LABORATORY

No. 15

南西海区水産研究所研究報告

第 15 号

昭和58年3月
南西海区水産研究所
広島県大野町

NANSEI REGIONAL FISHERIES RESEARCH LABORATORY
FISHERIES AGENCY
ÔNO-CHÔ, HIROSHIMA-KEN JAPAN

March 1983

南西水研報
Bull. Nansei Reg. Fish.
Res. Lab. No 15, 1983

南西海区水産研究所研究報告

第 15 号

目　　次

別途発表業績

南西水研報
Bull. Nansei Reg. Fish.
Res. Lab. No. 15, 1983

飼育実験によるカタクチイワシの雌1尾当り年間産卵数の推定

高尾　亀次・岸田　達・上田　和夫

Number of Eggs of Japanese Anchovy Produced per Female per Year Estimated by a Rearing Experiment

Kameji TAKAO, Tatsu KISHIDA and Kazuo UEDA

Since 1978 the Nansei Regional Fisheries Research Laboratory has estimated the abundance of adult anchovy (*Engraulis japonica* (HOUTTUYN)), based on the egg census, in addition to the analysis of catch and effort statistics (TAKAO 1980). The average number of eggs spawned per female is indispensable for conversion of the abundance of eggs in the sea to number of the adult anchovy that produced them. Attempt was made to determine the number of ovarian ova. The long-lasting spawning activities over nine months in the Seto Inland Sea makes it difficult to obtain the number of spawned eggs per female based on merely the counts of ovarian ova. Furthermore the number of eggs in the ovary were found differing from area to area within the distribution range of the species.

The authors have commenced rearing experiments of adult anchovy in an outdoor tank of 2×2×3m to determine actual number of spawned eggs. Fish of 0-age group that hatched in the autumn 1980 were brought to the tank on 23 June 1981, and then kept until October 1982. The fish spawned continuously from late July to early November 1981 and again discharged eggs since April 1982. The death rate stayed at low-level below 0.02% per day except 15 to 18 August 1981 when over-feeding caused mass mortality of 11 to 65%. Nevertheless, the fish actively discharged eggs even on 17, 18 August just in the disastrous decrease in number.

During the period of three months since late July, the average eggs production per day per female varied from one to 1, 031, and the accumulated counts reach 15, 400 eggs. A detailed observation were conducted from 19:00 on 17 August to 06:00 on 18 August. The counts for every one-hours were less than ten eggs discharged on the preceeding day before 22:00 and increased since then, having reach a peak of 1, 500 newly spawned eggs at 23:00. After midnight the eggs decreased in number, having dropped to 40 at 04:00 and about 300 at 05:00 and 06:00. The data indicate that the anchovy discharge eggs most actively at 22:00 to the midnight.

The long-lasting spawning of the same school in the tank confirms that a single fish spawns more than one batch of a year, even if the activity of reproduction varies remarkably from

1982年11月1日受理，南西海区水産研究所業績　第126号，〒739-04　広島県大野町

season to season. The anchovy eggs collected by plankton nets are found most abundant in June in the nearby spawning grounds of the Hiroshima Bay. Thus the accumulated count of 15, 400 eggs per female obtained from the rearing experiment that started on 23 June is definitely an underestimate of the average fecundity of the species. Taking the seasonal change of egg abundance in the sea and the duration of the rearing experiment into account, the authors propose that a female anchovy produces about 21, 000 eggs per year.

高尾（1980）が1978年から行っているカタクチイワシ親魚量推定においては，漁獲統計の解析による方法のほかに卵分布量にもとづく方法も併用している。卵分布量を親魚量に換算する際に用いる雌1尾当りの平均産卵数については，十分な知見が得られていなかったため，従来，卵巣内卵数つまり抱卵数が用いられてきた。カタクチイワシの抱卵数については，TAKESHITA and AIKAWA（1960）が日向灘で，宇佐美・杉山（1962）が陸奥湾で調査しているが，体長11cm以上では前者は卵径0.4mm以上で約3,500～4,200粒，後者は卵径0.8mm以上で6,000～26,000粒となっている。瀬戸内海においては，井上（1949），前川・八柳（1951），巻田（1954）が抱卵数を求めているが，その推定値は体長8cm前後で2,500～18,000粒である。いっぽう，カタクチイワシは卵巣内卵の卵径が多峯型の度数分布をしている（浅見1953，巻田1954，相川1954）ことから多回産卵と想定される。したがって，雌1尾が海中に放出する卵数を決定するには抱卵数のみでは不十分である。筆者らは産出卵数の知見を得るために1980年から親魚の飼育を行ってきた（高尾・岸田 1981）。1981年には長期飼育に成功し，海中に放出した卵数について知見を得たので報告する。

本報告のとりまとめに当っては南西海区水産研究所企画連絡室長林繁一および同内海資源部長尾形哲男両氏に原稿を校閲していただいた。また日本海区水産研究所企画連絡室長服部茂昌氏には貴重な助言をいただいた。記して深謝の意を表する。

材　料　と　方　法

1981年6月23日，大竹市阿多田漁業協同組合所属のいわし機船船びき網漁船が広島湾で漁獲したカタクチイワシ活魚約1,000尾を調査船せと（5.6ﾄﾝ）で当研究所に輸送し，屋外20ﾄﾝ水槽に収容した。水槽収容後3日以内に半数以上が死亡し，飼育実験に供したのは326尾であった。

斃死魚のうち130尾を測定した結果，体長範囲7.2～9.3cm，平均体長7.8cm，平均体重4.6g，平均脊椎骨数44.94であった。

産卵開始は1981年7月12日で32粒，その後は途切れて7月22日に283粒が採集されたのみであった。継続的な産卵が認められたのは7月31日からで11月2日までの95日間続いた。その後は水温の低下と共に年内の産卵を休止した。1982年4月30日以降に再度産卵を始め，7月現在もなお約30尾が生残って産卵を継続している。本報告では1981年7月31日から11月2日までの資料を用いた。

カタクチイワシの雌雄を外観から区別することは困難である。したがって飼育群のなかの雌の尾数は，斃死魚が生じた都度判定した雌雄個体数の累計値から性比を求め推定した。

飼育海水には当研究所沖合40mから常時ポンプで汲み上げたものを使用した。海水はゴミや天然卵の混入を防ぐためにＮＧＧ52のネットで沪過され１日当り約100トンが使用された。

毎日９時に投餌，卵採集，および天候と表面水温の測定を行ったが，日曜日および休日の一部には欠測した。なお塩分については1980年の予備調査で20～30‰の範囲内で変動幅が小さいことが判っていたので（高尾・岸田1981）測定しなかった。

卵は，表・中層からはサイフォンで海水とともに集めて寒冷紗でこし，底層からは飼育水槽の排水口にネットを装置して採集した（Fig. 1)。集められた卵は翌朝回収して計数し，飼育魚の総産卵数とした。日曜日と月曜日および欠測した休日と翌日の各採集卵数はそれぞれ２等分した。１回の卵数が1,000粒までは全数をかぞえ，それ以上の場合は，１ccのステンペルピペットにより６～10回の計数を行いその平均値を用いて卵数を推定した。

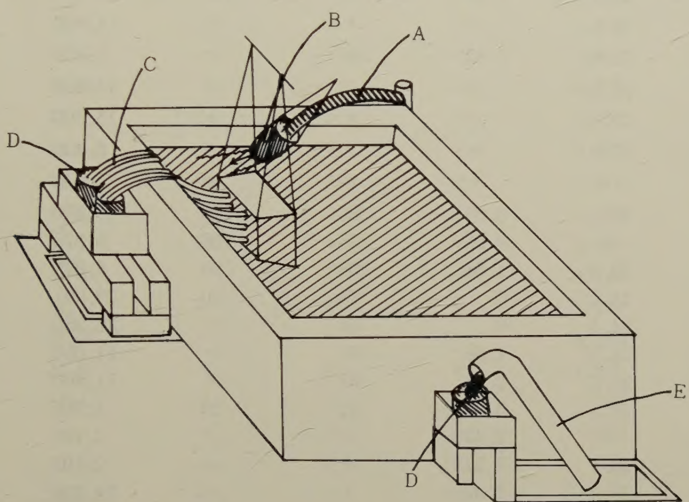


Fig. 1 Rearing tank of anchovy and apparatus for egg collection (after TAKAO and KISHIDA 1981).
A: Inlet of sea water B: Filter net
C: Syphones for surface egg collection
D: Egg collection nets E: Control pipe for water level

産卵時刻を知るため，８月17日19時から８月18日６時まで連続観測を行った。この観測では20時から２時までは30分間隔，それ以前および以後は１時間間隔で表・中層から卵を採集した。卵の発育期区分は服部（1958）による。

なお天然における産卵状況については1981年に広島県水産試験場が実施した卵稚仔分布調査の資料を用いた。

結　　　果

1. 飼育経過

Table 1に継続的に産卵を開始した７月31日からの日別水温，１尾当り投餌量，生残り親魚尾数，

Table 1 The records spawning in the captivity for anchovy from July 31 to November 2, 1981.

Date	Water temp. at 0900 (℃)	Food per fish (g)	Number of adults	Estimated number of female (a_i)	Number of eggs collected (b_i)	Average egg counts per female ($G_i = b_{i+1}/a_i$)
July 31	23.8	4.10	293	124	3,256	26
Aug. 1	22.5	2.05	〃	〃	11,459	92
2	—	3.75	〃	〃	3,383	27
3	23.2	4.10	〃	〃	3,373	27
4	23.6	4.12	291	〃	8,660	70
5	22.5	〃	〃	〃	8,180	66
6	22.7	〃	〃	〃	2,835	23
7	22.1	3.79	290	〃	8,944	72
8	22.7	3.81	289	123	15,150	123
9	23.2	〃	〃	〃	15,900	129
10	22.8	3.82	288	〃	5,350	44
11	22.5	〃	〃	〃	13,830	112
12	22.4	〃	〃	〃	15,400	125
13	22.9	〃	〃	〃	8,400	68
14	—	〃	〃	〃	15,026	122
15	23.1	3.85	286	122	13,800	113
16	—	4.66	236	106	6,875	65
17	23.9	1.68	208	95	6,875	72
18	24.2	0	73	30	29,901	997
19	24.4	0	65	〃	26,260	875
20	24.5	1.56	64	〃	11,050	368
21	24.3	2.38	63	〃	11,800	393
22	24.5	3.22	62	29	4,365	151
23	—	2.42	〃	〃	2,105	73
24	23.7	3.22	〃	〃	2,105	73
25	24.3	2.42	〃	〃	24,700	852
26	24.4	2.10	〃	〃	20,000	690
27	23.7	1.61	〃	〃	25,950	895
28	23.2	〃	〃	〃	29,910	1031
29	23.6	〃	〃	〃	16,080	555
30	—	〃	〃	〃	8,040	277
31	24.1	〃	〃	〃	8,040	277
Sep. 1	24.6	〃	〃	〃	7,020	242
2	24.5	〃	〃	〃	4,288	148
3	24.2	〃	〃	〃	4,736	163
4	24.7	〃	〃	〃	2,752	95
5	23.4	〃	〃	〃	7,800	269
6	—	〃	〃	〃	4,048	140
7	23.4	〃	〃	〃	4,048	140
8	23.7	〃	〃	〃	9,308	321
9	23.8	〃	〃	〃	5,920	204
10	23.2	1.94	〃	〃	3,801	131
11	22.9	1.61	〃	〃	3,360	116
12	22.7	〃	〃	〃	4,160	143
13	—	2.26	〃	〃	864	30
14	22.7	〃	〃	〃	864	30
15	—	—	〃	〃	2,928	101
16	22.0	2.42	〃	〃	2,928	101

(continued)

Date	Water temp. at 0900 (℃)	Food per fish (g)	Number of adults	Estimated number of female (a_i)	Number of eggs collected (b_i)	Average egg counts per female ($G_i = b_{i+1}/a_i$)
Sep. 17	22.1	2.26	62	29	2,544	88
18	22.3	〃	〃	〃	6,762	233
19	22.4	〃	〃	〃	5,092	176
20	—	〃	〃	〃	876	30
21	22.7	〃	〃	〃	876	30
22	22.7	3.23	〃	〃	3,857	133
23	—	—	〃	〃	2,591	89
24	22.9	2.26	〃	〃	2,591	89
25	23.0	2.10	〃	〃	344	12
26	23.0	2.26	〃	〃	1,976	68
27	—	—	〃	〃	2,840	98
28	22.4	2.30	61	〃	2,840	98
29	21.7	〃	〃	〃	4,864	168
30	21.6	〃	〃	〃	1,690	58
Oct. 1	22.1	1.64	〃	〃	1,632	56
2	22.1	〃	〃	〃	5,872	203
3	21.9	2.30	〃	〃	3,918	135
4	—	—	〃	〃	3,475	120
5	21.7	1.64	〃	〃	3,475	120
6	21.4	〃	〃	〃	3,146	109
7	21.7	〃	〃	〃	1,739	60
8	21.4	〃	〃	〃	4,149	143
9	21.5	〃	〃	〃	4,845	167
10	21.4	〃	〃	〃	239	8
11	—	〃	〃	〃	628	22
12	20.9	〃	〃	〃	628	22
13	20.8	〃	〃	〃	15	1
14	20.9	〃	〃	〃	1,442	50
15	20.5	〃	〃	〃	7,192	248
16	20.6	〃	〃	〃	6,125	211
17	20.5	〃	〃	〃	2,562	88
18	—	—	〃	〃	1,454	50
19	21.0	1.64	〃	〃	1,454	50
20	20.4	〃	〃	〃	1,941	67
21	20.3	〃	〃	〃	2,758	95
22	20.7	1.67	60	〃	2,722	94
23	19.8	〃	〃	〃	2,255	78
24	19.4	〃	〃	〃	887	31
25	—	—	〃	〃	793	27
26	18.8	1.67	〃	〃	793	27
27	18.9	1.33	〃	〃	36	1
28	17.0	〃	〃	〃	243	8
29	19.4	1.67	〃	〃	9,125	315
30	18.2	〃	〃	〃	1,930	67
31	18.5	2.17	〃	〃	920	32
Nov. 1	—	—	〃	〃	613	21
2	17.7	2.50	〃	〃	613	21

採集卵数，1尾当り平均産卵数を示す。飼育魚は収容直後には餌をとらなかったが6月26日から摂餌を開始した。餌は主に冷凍オキアミを用い，1ヵ月に5～10回はイカナゴすり身を計1日1尾当り1g前後投与し，飼育開始32日後の7月29日からは3.75～4.66gに増加させた。その後17日を経過した8月15日から大量の斃死が起った。この時期の日別死亡率は8月15日17%，8月16日12%，8月17日65%，8月18日11%を示した。8月17日には投餌量をそれまでの½以下の1尾当たり1.68gに減らし，翌18，19日には投餌を中止した。8月20日からは1日1尾当りの投餌量を約2gとして飼育を継続したが，その後は8月20日，21日，9月27日，10月21日に各1尾斃死したのみであった。飼育水温は8月15日，16日には若干昇温して24℃前後となったが，この程度の温度は7月31日および8月19～26日，8月31～9月4日にも現われている。斃死魚の胃が膨満していたことから，死亡の主因は過剰投餌と昇温の複合作用によるものと考えられる。

2. 雌1尾当り日別産出卵数

日別総産出卵数（b_i）の最高値は8月28日の29,910粒，最低値は10月13日の15粒であった。この間に産卵に関与していた雌の推定尾数（a_i）は124尾から29尾に減少しているので産卵量の日別変化は雌1尾当り平均産出卵数 $G_i = b_{i+1}/a_i$ によって比較する必要がある。雌1尾当り平均産出卵

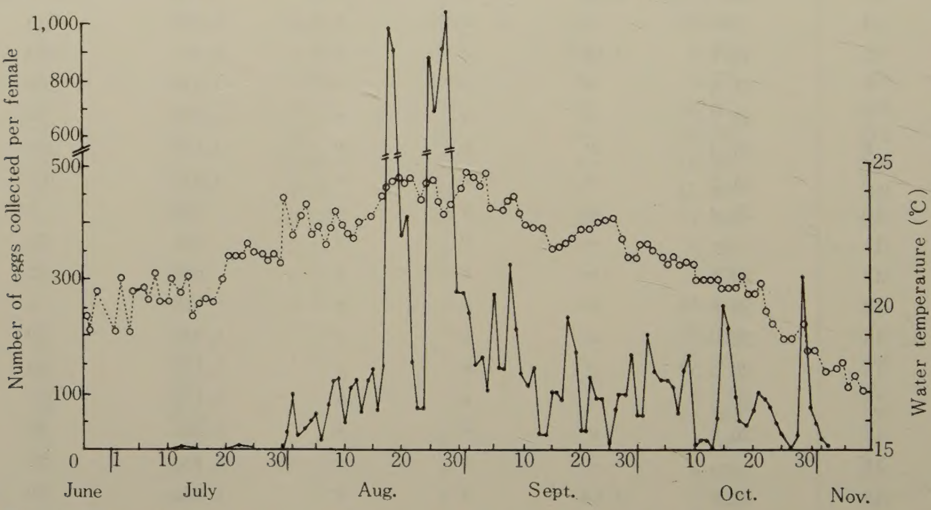


Fig. 2 Water temperature in the rearing tank and number of collected eggs per female during June 26 through November 7, 1981.
Closed and open circles denote number of eggs and water temperature, respectively.

数をFig. 2に示した。この値は8月28日の1,031粒から10月13日の1粒の間に広がっている。全期間をつうじての雌1尾1日当りの平均産出卵数は，162粒，標準偏差214粒，変動係数132%であった。さらに細かくみると7月31日～8月16日は23～125粒で少なかった。大量斃死が起った8月17，18日にはそれぞれ997粒，875粒を記録して第1のピークを示した。その後5日間は400粒以下に低下したが，8月24～27日には再度690～1,031粒と増加して第2のピークを示した。9月になって水温は下降期に入ったが，第2のピーク以降の産卵数は，およそ300粒以下の範囲で増減を繰返した。8月29日以降 $G_i \geqq 200$ の日は12日，$200 > G_i \geqq 100$ の日は18日，$G_i < 100$ の日は36日であった。

このように，1尾当り産出卵数は，8月中～下旬にピークを示したがその後は減少し，小さな増減を繰返しながらも産卵は長期間継続することが知られた。全産卵期間内の雌1尾当り総産出卵数は，15,400粒と推定された。

3. 産卵時刻

8月17日19時から18日6時までの時間毎の採集卵数をFig.3に示す。採集卵数は19時から21時までは10粒以下で，後期卵（stage C）が多かった。22時から急激に増加し，22時に220粒，23時までに1,536粒，0時までに720粒を示した。その後は減少し1～3時には243～480粒，4時には僅か40粒，5～6時には300粒であった。22時以降の採集卵は殆んど前期卵（stage Aa*）であった。

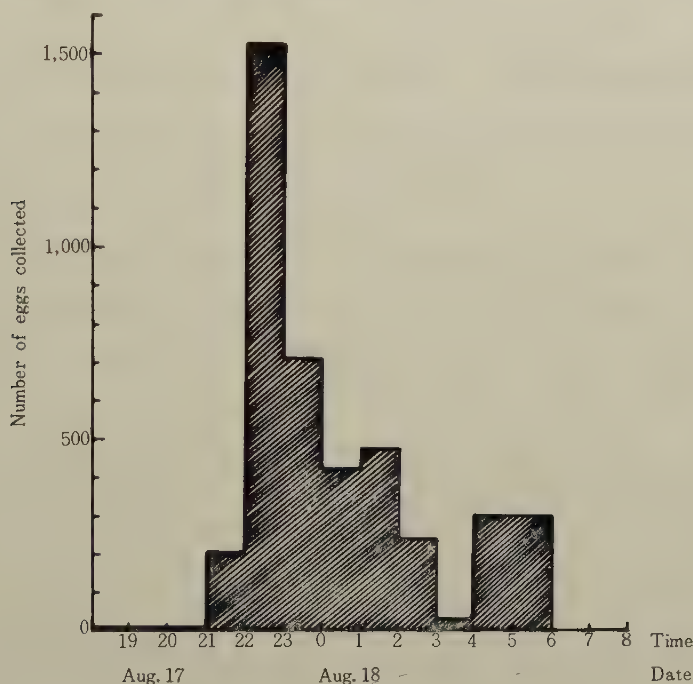


Fig. 3 Number of eggs collected from surface layer of the rearing tank at one-hour interval, 1900 on 17 to 0600 on 18 August 1981.

考　　察

1. 産卵の回数と条件

Fig.2に示すとおり本実験におけるカタクチイワシの継続産卵期間は7月31日から11月2日に至る95日間であった。特に雌が僅か30尾に減少した8月18日以降も引続いて卵が採集されていることから，カタクチイワシはこの期間中何回にも分けて産卵していると考えることができよう。カタクチイワシの多回産卵については，これまで卵径組成に3～4個のピークがみられることから推定されていた（浅見1953，巻田1954，相川1954）。今回の実験では日別1尾当り平均産出卵数は8月18

＊前期卵（A-stage）は Aa, Ab および Ac に細分される 服部（1958）.

日，19日，25～29日に555～1,031粒の著しいピークを記録したほかは多い場合でも300粒程度にとどまっている。このことは，このピークの時期には多くの雌が産卵行動に参加したか，あるいは1回の放卵数が多かったか，あるいはその両方が作用したか，3通りの可能性が考えられる。

産卵と水温との関係をみると産卵開始時は23.8℃を示した。その後はしばらくの間22.1～23.6℃で推移し，最大の産卵ピークを示した8月中～下旬には23.2～24.4℃を示した。その後は水温は徐徐に低下し，産卵終期では17.7℃であった。このように本種はほぼ18℃以上で産卵を行っている。

なお，飼育に供した成魚は，高尾（1964）によれば前年の秋生れ群と推定された親魚群である。この群は従来の見解では発生の翌年の6～7月に産卵し，産卵後は逸散もしくは死亡すると考えられていた。今回の結果からは11月上旬まで産卵を継続し，さらに翌年2年魚となって春から夏にかけても産卵することが確認されたが，これらの詳細については稿をあらためて報告する予定である。

1981年に広島県水産試験場が毎月上旬に実施した広島湾内14定点の卵採集結果をTable 2に示す。㊕ネット垂直びきによる1定点当り卵採集数は，6月に220粒できわめて高く，ついで7月に105粒を記録したが，5月および8～11月では37粒以下できわめて少なく，1～4月と12月は皆無であった。本実験供試魚は6月23日に採集されたが，この群と広島湾の産卵親魚群は同一系群と想定されるので，供試魚群は採集される以前での5～6月ごろすでに産卵を始めていたことが予測される。水槽に収容後1カ月以上もの間産卵が殆んど行われなかったのは，収容後わずか3日間に半数以上が死亡したことから考えて，人為的に加えられた傷害や環境の変化によってストレスをひきおこしたためで，環境に馴化してきた7月末から再度産卵を開始したものと考えられよう。

Table 2 Number of anchovy eggs collected at 14 stations in the Hiroshima Bay, April to December, 1981.

Month	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
Total number of eggs	0	38	3076	1465	521	435	277	17	0
Egg counts per tow	0	2.7	219.7	104.6	37.2	31.3	19.8	1.2	0

2. 雌1尾当り産出卵数

7月31日から11月2日の間における雌1尾当りの産出卵数は約15,400粒であった。今回の実験期間が全産卵期を包含しているとすればこの値は年内総産出卵数とみなすことができる。しかし前述のように自然界では6月を中心に産卵が行われているので，今回の飼育実験によって求めた値は過小評価となっている可能性がある。広島水試の調査結果によると，5，6月の採集卵数と7月以降の採集卵数の比は1.2：1となっている。一方，親魚量は漁獲，逸散，死亡などの要因により時間の経過と共に減少していく。大阪湾のカタクチイワシ資源解析の報告（土井他 1979）によると，資源量の変動傾向から推定された最も妥当な旬別生残率は5月中旬～7月上旬0.61，7月中旬～8月中旬0.48，8月下旬～10月上旬0.76となっている。広島湾では漁獲効率の高いまき網漁業が皆無のため，旬別生残率をこれより高い0.8と仮定して5，6月の各月平均親魚量の和に対する7～11月の各月平均親魚量の和の比を求めると3：1になる。したがって，6月以前と7月以降の1尾当り累積産出卵数の比は0.4：1となる。これを用いて実験値を修正すると1尾の年内にお

ける総産出卵数は約21,000粒と計算される。高尾（1980）は，前川・八柳（1951），巻田（1954），井上（1949）の報告により，瀬戸内海の６～７月におけるカタクチイワシ卵巣内の長径0.6㎜以上の熟卵および完熟卵と思われる卵数を5,000粒と仮定したが，今回の結果では，産卵期間中の雌１尾当り総産卵数はその値の４倍以上になることを示した。更に１日ごとの総採集卵数をみると，雌の数が30尾に減少した後の９，10月期には，１日の総採集卵数が5,000粒を下回っている日が多い。このことは成熟に達したおよそ5,000粒の卵群は１度には放卵されないことを示している。したがって，個々の雌の産卵は何回にもわたって行われていると考えられる。なお，今回の飼育実験で求めた産卵数はサイフォンで採集した卵の数であるので，回収される前に親魚に食べられて過小評価されている可能性がないわけでもないが，産出された大多数の卵は速やかに夜のうちにネットに採集されていると思われる。

今回の実験ではオキアミおよびイカナゴのすり身を与え，その投与量は当初１尾当り１ｇからやがて３～４ｇ，大量斃死後は１～２ｇとした。天然ではカタクチイワシはおもに動物性プランクトンを摂っているとされており，また天然魚の成長速度が飼育魚ほど速くないことから，今回の飼育における餌の質を含む総投与量は天然のそれよりもかなり多かったものと考えられる。

Nikolsky（1965），Bagenal（1969）は産卵数は食物供給量によって変化すると指摘している。Bagenal は brown trout の飼育実験を行い，食物条件のよいものほど抱卵数が多い傾向があると述べている。したがって実験から求めた産出卵数が天然の場合とは異なる可能性もあるので，この点に関してはさらに検討を行う予定である。

3. 産卵時刻

天然海域における本種の産卵について，中井（1955），山中・伊東（1957）は20～０時の前夜

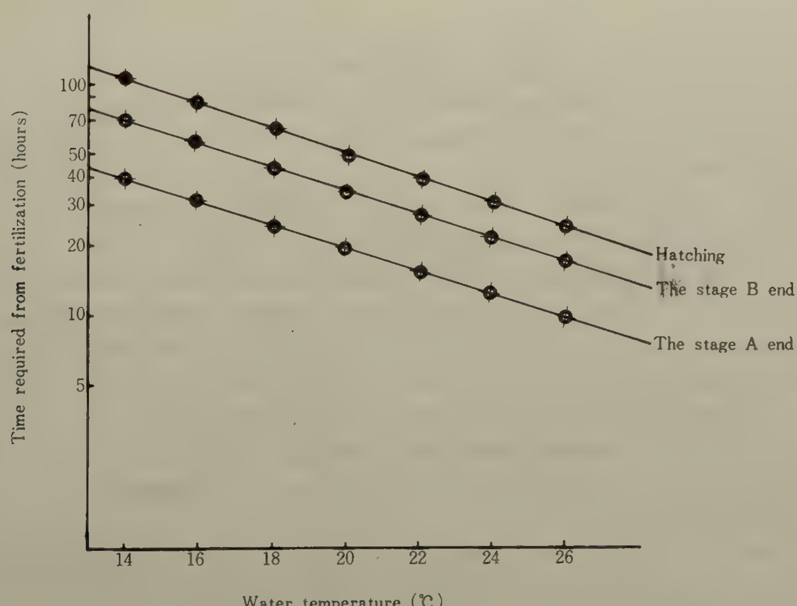


Fig. 4 Relation of development of anchovy eggs and water temparature after Hattori (in press).

半，山田（1958）は23～4時の主として後夜半に行われると述べている。

飼育条件下での本種の産卵は，21時までは後期卵（stage C）が多く，その後の採集卵は殆んどが前期卵（stage Aa）であった。服部（1982）の卵発育速度と温度との関係についての資料を Fig. 4 に示す。今回の飼育期間中の産卵数の多い時期の平均水温は22～24℃であり，この水温では，服部の資料によれば中期卵（stage B）に達するまでの経過時間は12～15時間とされるので，後期卵（stage C）は前々夜のものと推定される。また0時以後はそれ以前に産出した卵の回収もれも含まれるから，産卵は主として22時～0時の前夜半のきわめて短時間に行われると考えてよい。

摘　　要

カタクチイワシ成魚の飼育実験を行い，産卵行動や産卵数について以下の結果を得た。

1）飼育した成魚は前年の秋生れ群と推定される。従来，この群は発生した翌年の6，7月に産卵すると考えられていたが，秋まで産卵することが明らかになった。飼育期間中の主たる産卵時期は7月31日から11月2日までであった。

2）飼育に供した群は，3ヵ月以上にわたって産卵を持続した。また集団としての産出卵数は季節によって大きく異った。

3）本種の産卵時刻は22時～0時の前夜半と推定された。

4）年間における雌1尾の産出卵数は21,000粒前後と推定された。

文　　献

浅見忠彦，1953：イワシ類の卵巣卵に関する研究，日水誌，**19**（4），398～404.

相川広秋，1954：カタクチイワシの資源生物学的研究，九大水産学教室謄写版，1～20.

Bagenal, T. B., 1969: The Relationship Between Food Supply and Fecundity in Broun Trout *Salmo trutta*. L., J. Fish. Biol., 1, 167～182.

土井長之・高尾亀次・石岡清英・吉田俊一・林凱夫・辻野耕実・鍋島靖信　1979：カタクチイワシの資源解析，関西国際空港漁業環境影響調査報告，第3分冊漁業生物編（上），794～805.

服部茂昌，1958：海産魚の卵稚仔査定の手引き，東海地区水産資源担当者研修会，10～11.

———，1982：カタクチイワシ卵の発育速度と温度との関係，南西海区ブロック内海漁業研究会報告(印刷中)。

井上　明，1949：洲本附近の漁獲カタクチイワシについて，日水誌，**15**（8），385～390.

前川兼佑・八柳健郎，1951：カタクチイワシの生態学的研究，日水誌，**16**（12），174～178.

巻田一雄，1954：大阪湾重要魚類生態調査1，カタクチイワシ，大阪水試報，1～20.

中井甚二郎　1955：昭和24～26年鰮資源協同研究経過報告，東海水研，1～84.

Nikolsky, G. V., 1965: Fish Population Dynamics (Translated from Russian by J. E. S. Bradley), Oliver and Boyd, Edinburgh, 323 pp.

Takeshita, K. and H. Aikawa, 1960: Biology of Japanese Anchovy in the Nankai Region, (11), Records Oceanogr. Wks. Japan. Spec. NO. 4, 159～164.

高尾亀次，1964：瀬戸内海のカタクチイワシ *Engraulis japonica*（Houttyn）の生態について，内海水研報C輯，2，1～50.

————・岸田達，1981：カタクチイワシ飼育環境下における産卵状態，第13回南西海区ブロック内海漁業研究会報，53～57.

宇佐美修造・杉山久治，1962：カタクチイワシ *Engraulis japonica* （HOUTTUYN）の再生産力について—I. 陸奥湾におけるカタクチイワシ卵巣卵の卵径組成からみた成熟と卵数，東海水研報37，1～9.

山中一郎・伊東祐方，1957：昭和29年鰮資源協同研究経過報告，日水研，91.

山田鉄雄，1958：大村湾内カタクチイワシの産卵生態，長崎大学水産学部研報6，73～82.

南西水研報
Bull. Nansei Reg, Fish.
Res. Lab. No 15, 1983

豊後水道域における魚群分布

花岡藤雄

Distribution of Fish Schools in the Bungo Suido (Channel) Determined by Acoustic Surveys.

Fujio HANAOKA

The Bungo Suidō is one of the two major channels connecting the Seto Inland Sea to the Pacific Ocean. It comprises migratory and drifting routes of commercially important fishes, and their eggs, larvae and juveniles. In order to understand the behavior of fish schools, acoustic surveys were conducted employing four research vessels equiped with echo sounders for commercial fishing operation in March 1972. The four vessels cruised along eight lines set so as to cover the channel.

The echo sounders recorded dense accumlation of fish schools in boundary areas between the inland sea water and the offshore water identified as such by the salinity of or below 34.5‰ at 30m depth layer in the month when the southward movement of inland sea water prevailed. The vertical profiles of acoustic record showed that the fish avoided the inflowing offshore water of relatively high temperature above 18℃. Shape of the records indicates that anchovy, *Engralulis japonica* (HOUTTUYN), and trigger fish, *Navodon modestus* (GÜNTHER), represented major components of the fish schools discovered therein.

Similar acoustic surveys were repeated in the same area in September 1972, when the southward flows were weakend the immigrant fishes from the open sea aggregated therein. At this occasion, the vessels covered five blocks instead of lines. The fish were found densely distributed in the inland sea water and in the coastal water again represented by 34‰ of salinity at 30m depth layer. Analyses of records implied that dominant species were anchovy, round herring, *Etrumeus teres* (DEKAY), mackerels, *Scomber japonica* HOUTTUYN, and *S.australasicus* CUVIER, and trigger fish. The fish appeared aggregating again in the relatively cold coastal water below 24℃.

At the both occasions, the fish schools in densely distributed areas were found not only large in size but also narrow in distance between them. The tidal current were found affecting distribution of fish which decreased in the swift flow of about four knots in narrow strait.

1982年11月11日受理　南西海区水産研究所業績127号，〒739-04　広島県大野町
住所　南西海区水産研究所高知庁舎，〒780　高知市桟橋通り

豊後水道は紀伊水道とともに瀬戸内海系水と外洋系水との混合域にあたり，内海と外海にまたがって生活するイワシ類等の回遊路で，卵・稚仔魚の補給路でもある（横田1953，浅見1962，能津1965）。

この水域では水道中央部及び東部を通って外洋系水が流入することが多く，その勢力は春から夏にかけて強い。内海系水は主として西部沿岸域を通って流出し，とくに秋から冬にかけて卓越する。この流れに加えて固有の強い南北方向の潮流があり，春秋の最強時には北部の速吸瀬戸で４―５ノット，南部の鶴見崎―由良崎間で１―２ノットに達する（海上保安庁1967）。

潮流によって起る瀬戸内海系水と外洋系水との混合がひき起す豊後水道の海洋構造の時空間変化，及びそれに伴う魚卵・稚仔魚，魚群分布の変化は漁業にとって重要な関心事である。そのため，1971年４月から1973年３月にかけて，昭和46，47年度科学技術庁特別研究促進調整費による「豊後水道海域における海洋環境と海洋生物生産機構の研究」が実施された。ここではこの協同研究で得られた資料を用いて，イワシ類等の1972年３月及び９月における魚群分布と，それらが潮流によって起る短期の変化について報告する。

本文に入るに先立ち，海上での調査に当られた愛媛県水産試験場試験船よしゆう丸，大分県水産試験場試験船速吸丸，遠洋水産研究所調査船俊鷹丸，南西海区水産研究所調査船しらふじ丸及びこたか丸の乗組員，調査員各位に厚くお礼申し上げる。この研究を進めるについて，西海区水産研究所浅見忠彦企画連絡室長（当時当研究所外海資源部長），当研究所尾形哲男内海資源部長（当時外海資源部長），古藤力同第一研究室長には終始ご指導をいただいた。当研究所桑谷幸正所長，林繁一企画連絡室長，佐野蘊外海資源部長にはこの原稿を注意深く読んでいただいた。ここに記して厚くお礼申し上げる。

調査及び解析の方法

本研究のための海洋観測と魚群調査は1972年３月６―15日と９月７―15日に実施された。３月には大分県芹崎，愛媛県由良崎から速吸瀬戸の間に５浬間隔で６定線を設け，さらに伊予灘に向って２定線を設定した（Fig. 1）。９月には伊予灘を除き豊後水道内で３月と同様の定点を設定した(Fig. 2)。今回の調査に参加した俊鷹丸，しらふじ丸，こたか丸，よしゆう丸，速吸丸の魚群探知機は漁船に広く用いられているもので，その型式や主要性能等は **Table1** に示すとおりである。

各調査船の航程を **Table 2, Table 3** に示す。３月の調査では第１日（３月６日）に俊鷹丸，よしゆう丸の２隻が **St. 11―16,** しらふじ丸，速吸丸の２隻が **St. 21―25** をそれぞれ２時間ずつの間隔で西から東に向けて調査した。第２日には俊鷹丸，速吸丸が，第３日にはよしゆう丸，しらふじ丸がそれぞれ上記の２定線を東から西に向けて調査した。第３，４日には **St. 31―35, St. 41―45** をやはり２隻ずつまず西側から，ついで東側から調査し，第５，６日には豊後水道北部にある **St. 51―55, St. 61―66** を調査するとともに俊鷹丸，よしゆう丸は第３，４日伊予灘における２つの定線の調査を行なった。この方式によって同一定点における時空間変化を調べるよう企図したが，荒天等のため一部の船の運航が妨げられた（**Table 2**)。

９月の調査では34定点を **Fig. 2** に示した**A,B,C,D,E** の５ブロックに分け，１日１ブロックず

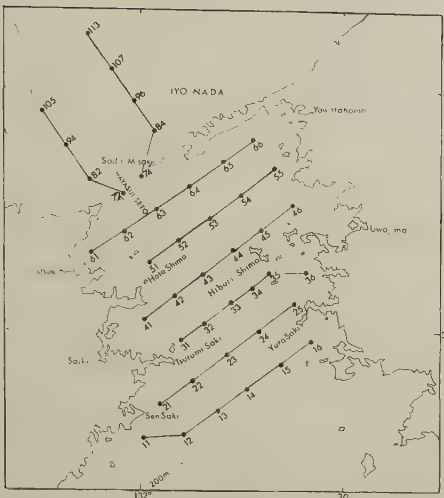


Fig. 1 Oceanographic stations occupied in March 1972.

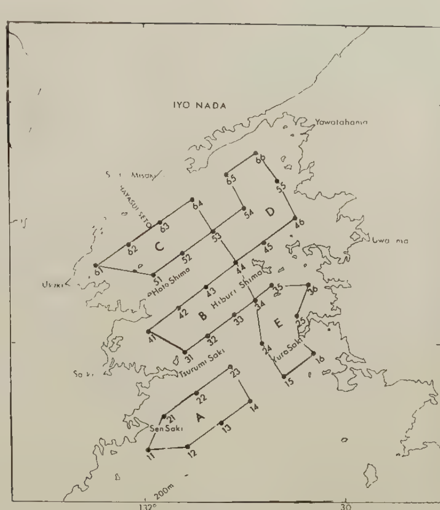


Fig. 2 Oceanographic stations occupied in September 1972.

Table 1 Echo-sounders on board of the reseach vessels.

Name of vessels	Gross tonnage	Echo-sounders		
		Type	Cycles	Depthrange
SHUNYŌ-MARU	185.9	SANKEN-TL-32	28KHz	0-100m
SIRAFUJI MARU	93.7	KAIJŌDENKI-B	24KHz	0-160m
KOTAKA-MARU	47.5	FURUNODENKI-FHG	28KHz	0-100m
YOSHŪ-MARU	68.0	KAIJŌDENKI-B	24KHz	0-160m
HAYASUI-MARU	69.8	KAIJŌDENKI-FGS	14KHz	0-160m

Table 2 Sequences of observations conducted by four reseach vessels, 6—15 March 1972.

Date \ Vessel	SHUNYŌ-MARU	SHIRAFUJI-MARU	YOSYŪ MARU	HAYASUI-MARU
Mar. 6	st. 11→ 12→ 13 → 14→ 15→ 16	st. 21→ 22→ 23 → 24→ 25	st. 11→ 12→ 13 → 14→ 15→ 16	st. 21→ 22→ 23 → 24→ 25
〃 7	st. 16→ 15→ 14 → 13→ 12→ 11	———	———	st. 25→ 24→ 23 → 22→ 21
〃 8	———	st. 25→ 24→ 23 → 22→ 21	st. 16→ 15→ 14 → 13→ 12→ 11	———
〃 9	st. 31→ 32→ 33 → 34→ 35→ 36	st. 41→ 42→ 43 → 44→ 45→ 46	st. 41→ 42→ 43 → 44→ 45→ 46	st. 31→ 32→ 33 → 34→ 35→ 36
〃 11	st. 36→ 35→ 34 → 33→ 32→ 31	st. 46→ 45→ 44 → 43→ 42→ 41	st. 46→ 45→ 44 → 43→ 42→ 41	st. 36→ 35→ 34 → 33→ 32→ 31
〃 12	st. 61→ 62→ 63 → 64→ 65→ 66	———	———	———
〃 13	st. 66→ 65→ 64 → 63→ 62→ 61	———	st. 51→ 52→ 53 → 54→ 55	———
〃 14	st. 72→ 82→ 94 →105	st. 51→ 52→ 53 → 54→ 55	st. 55→ 54→ 53 → 52→ 51	st. 61→ 62→ 63 → 64→ 65→ 66
〃 15	———	st. 55→ 54→ 53 → 52→ 51	st. 74→ 84→ 96 →107 →113	st. 66→ 65→ 64 → 63→ 62→ 61

Arrows denote order of observations.

Table 3　Sequences of observations conducted by four reseach vessels, 7—15 September 1972.

Vessel / Date	SHIRAFUJI-MARU	KOTAKA MARU	HAYASUI MARU	YOSYU-MARU
Sept. 7	st.33→32→33→32 →33→32→33	st.62→63→62→63 →62→63→62→63	st.51→52→51→52 →51→52→51→52	st.42→43→42→43 →42→43→42→43
〃 10	st.43→44→34→33 →32→31→41	st.34→44→43→42 →41→31→32	st.41→42→43→32	st.34→33→32→31 →41→42→43
〃 11	st.63→64→53→52 →51→61→62	st.51→61→62→63 →64→53→52	st.61→62→63→64 →53→52	st.53→52→51→61 →62→63→64
〃 12	st.44→45→46→55 →66→65→54→53	st.54→53→44→45 →46→55→66→65	st.46→55→66→65 →54→53→44→45	st.66→65→54→53 →44→45→46→55
〃 13	st.16→25→36→35 →34→24→15	st.24→15→16→25 →36→35→34	st.36→35→44→34 →→24→15→16	st.36→25→16→15 →24→34→35
〃 15	st.21→11→12→13 →14→23	st.23→22→21→11 →12→13	st.11→12→13→14 →23→22	st.13→14→23→22 →21→11

Arrows denote order of observations.

つ調査した。このうち St. 34, 44, 53 の３点は２つのブロックに含まれるので各ブロックの観測点は７－８点である。各ブロックについて４隻の調査船が観測開始点をずらして４点ずつ同時に調査を行なった（Table 3)。この方式によって各観測点で２時間間隔で４回の観測を行なうよう船を運航し，その間の魚群分布の変化に及ぼす潮流の影響をみた。この他に潮流の速い速吸瀬戸に近い St. 62—63 及びその南側の St. 51—52, St. 42—43, St. 32—33 の各２点間を，９月７日の９時から16時までの７時間にわたり，各調査船が１時間毎に連続調査した。

各定点においては測深，各層水温・採水等の観測及び魚卵・稚仔魚，プランクトンの採集を行なった。各定点間を航走中は常時魚群探知機を作動させた。

魚群探知機の記録から魚群量指数，魚群の間隔，遊泳水深等を求めた。魚群量指数は横田他（1953）の方法で下記のように計算した。

$$si = Li \times Hi$$

$$S = \sum_{i=1}^{n} s_i = \sum_{i=1}^{n} (L_i \times H_i)$$

$$s = \frac{S}{n}$$

s_i；i 番目の群れの魚群量指数

S；ある調査線上又は海域の魚群量指数

$\bar{s}$；その調査線上又は海域における平均魚群量指数

n；その調査線上又は海域の魚群の数

L_i；i 番目の魚群の長さ，記録紙上の長さと船速から求めた実長（浬）

H_i；i 番目の魚群の厚さ，記録紙上の高さから換算した厚さ（m）

魚の種類は記録紙上の形状から判断した。魚群の分布に影響する環境の指標値としては水温を用いる場合が多い。しかし豊後水道では内海系水と外洋系水との間の温度差が小さく，等温線の水平分布では両水塊の境をはっきり区別できない。ここでは魚群の主分布層である30m層の塩分を用い

て水塊を区分した。

結　　　果

1.　魚群の水平分布

3月における魚群の水平分布を映像が鮮明であった俊鷹丸としらふじ丸の魚探記録を用いて述べる。ただし伊予灘東側はよしゆう丸の記録による。Fig. 3 によると魚群は豊後水道南部鶴見崎沖でもっとも多く，次いで速吸瀬戸北側，佐田岬南側に多い。魚群の少ない海域は豊後水道南部の St. 11—14, St. 21—23, 日振島周辺の中部域 St. 33—36, St. 43—46, St. 52—55 である。魚群が多い鶴見崎沖は水道の西側を南下する内海系水と南から差し込む外洋系水との境界域とみられ，魚群の主分布層である30m層の塩分は34.4—34.5‰である。これに対して外洋系水とみられる34.6‰等塩分線は豊後水道南西部から北東方向に差し込み，それに続く34.5‰等塩分線は中央部を北上する傾向を示しているが，これらの等塩分線で示される水帯の中では魚群は少ない (Fig. 3)。

9月における魚群の水平分布は，しらふじ丸，こたか丸，速吸丸，よしゆう丸による資料を用いて検討した。Fig. 4 には同一の定点間を4隻の調査船が1—2時間の間隔をおいて通過して得た

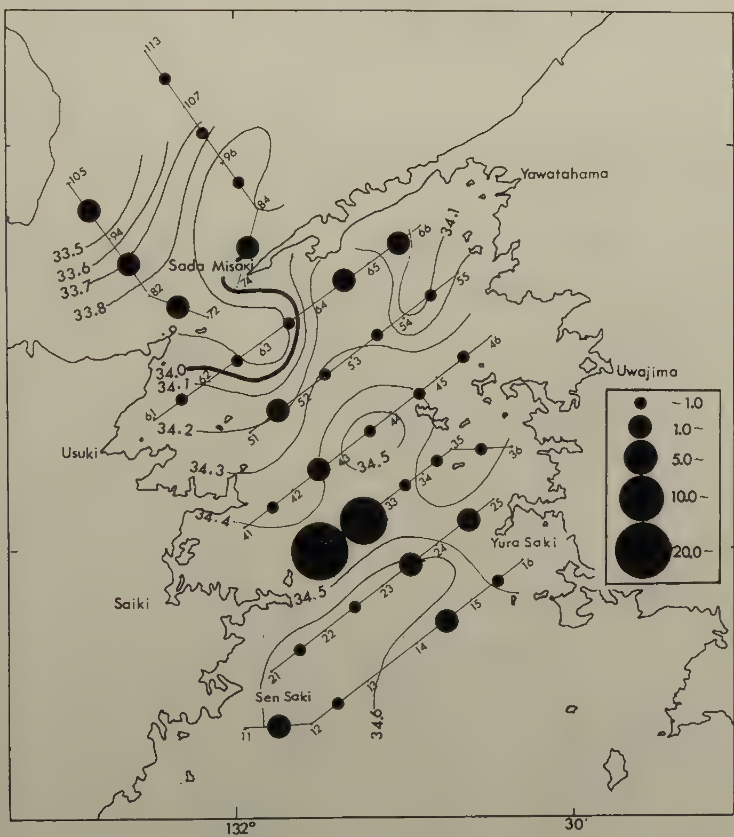


Fig. 3　Abundance indices along interstation lines, 6—15 March 1972.

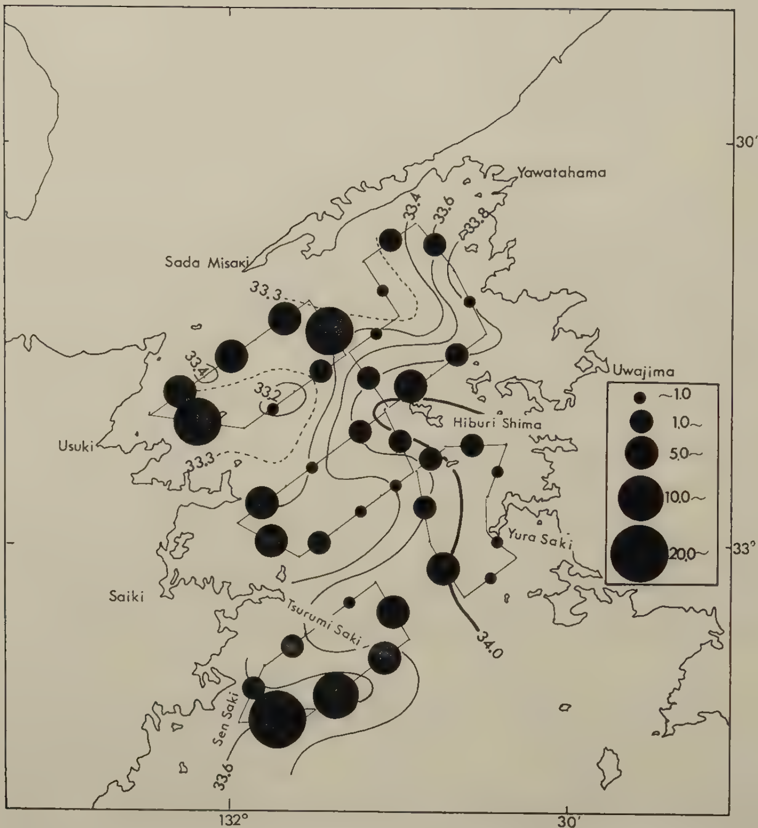


Fig. 4　Abundance indices along interstation lines, 10—15 September 1972.

4個の魚群量指数から1隻当り平均値を示した。その結果魚群は豊後水道南部芹崎沖の St.11—14 から北東方向にかけて34.0‰等塩分線に沿って多く分布し，速吸瀬戸南方の St. 53—64—62, 臼杵湾口の St. 51—61—62, 及び佐伯湾口の St. 31—41—42 にも多く分布している。水道南部の東側沿岸域の34.0‰以上の高かん域では魚群は少ない。又，St. 33 -43—52 を結ぶ線上及び佐田岬半島南方の St. 54—46 を結ぶ線上の比較的低かん水の張出しているところでも少ない。全般的には3月の調査時に比べて多い。

2.　魚群の鉛直分布

3月における魚群の鉛直分布を定線別に Fig. 5 に示す。魚群は18℃以上の外洋系水中には現れず，St. 11—13 では17—18℃の75m前後に認められ，その東側の St. 14—16 では16—17℃の40m層付近に分布していた。St. 22—25 間では比較的浅い10—30m層に見られている。この調査で魚群が最も多く，内海系水と外洋系水との境界域とみられる St. 31—33 では，15—15.5℃の50—60m層のやや深いところに分布している。その東側の St. 34—36 では14—15℃の30—40m層に多い。St. 41—46 間では14—16℃の10—20m層付近，St. 51—55 では13—14℃の20—40m層付近で魚群が記録された。速吸瀬戸に近く潮流の影響を最も強く受ける St. 61—66 の定線では，その南側の

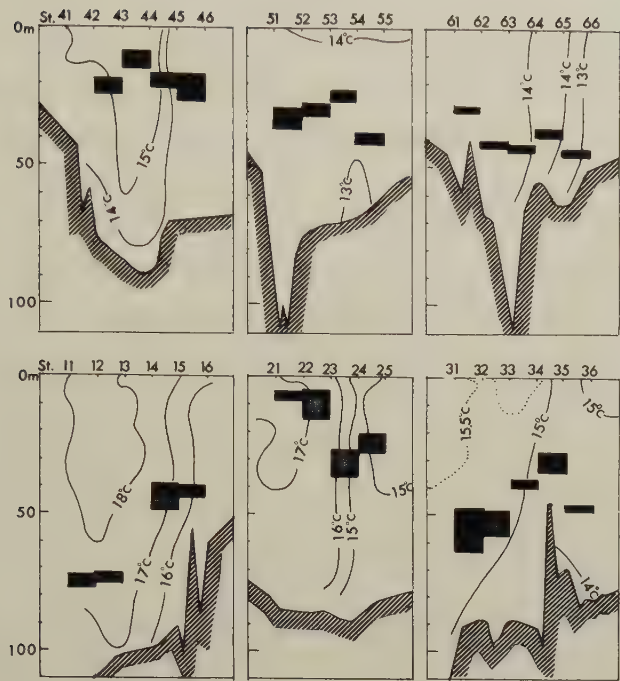


Fig. 5 Vertical distribution of fish-school along six interstation lines, 6—15 March 1972.

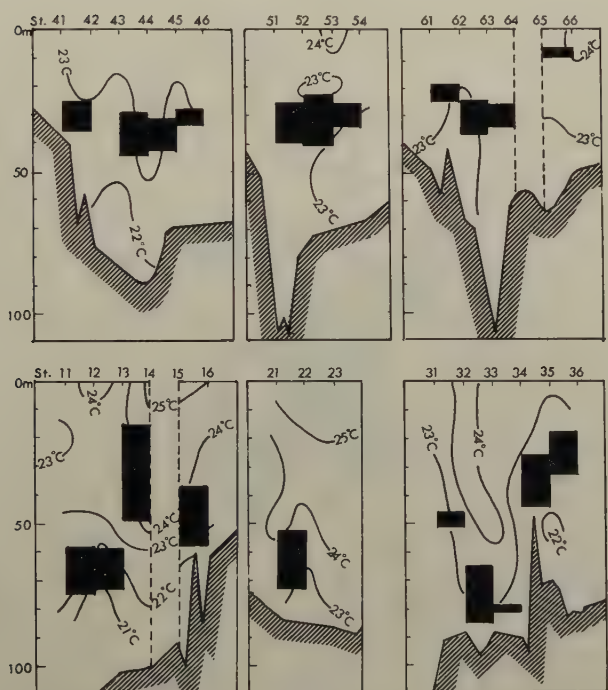


Fig. 6 Vertical distribution of fish-school along six interstation lines, 10—15 September 1972.

2定線に比べてやや深く30—50m層に分布していた。以上の観察から，魚群の分布層は鉛直混合の顕著な場所で深い傾向を示している。

9月は Fig. 2 に示したような各ブロック別の調査方式をとったので，3月のように東西方向線上の全定点間は調査されなかった。調査された定点間について，東西方向の魚群の鉛直分布を示すと Fig. 6 のとおりである。

魚群は全般に水温24℃以下のところに分布している。最南部の St. 11—16 間のうち St. 11—13 では23℃以下の60—70m層に，St. 13—14 では24℃前後の20—50m層に，St. 15—16 では24℃以下の40—60m層に分布している。St. 21—23 では23—24℃の50—70m層に分布し，24℃以上のところには認められない。St. 31—36 間では23℃等温線にそって，St. 31—32 で50m層，St. 32—34 で60—80m層，St. 34—36 で20—50m層に分布し，24℃以上のところには認められない。St. 41—46 及びSt. 51—54 ではいずれも23℃前後の20—40m層付近に分布している。速吸瀬戸に近い St. 61—64 でも23℃前後の20—40m層に分布し，その東側の St. 65—66 では23—24℃の10m層にみられる。全般的に3月に比べて分布する層が広い。

3. 群れの大きさとその間隔

魚群探知機に記録された個々の群れの大きさは多様である。3月に調査した各定点間における群



Fig. 7 Average size of fish-schools along interstation lines, 6—15 March 1972.

れの大きさの平均値を **Fig.** 7 に示す。魚群が多く分布している鶴見崎沖の **St.** 31—33 でとくに群れが大きい。記録形からみてこの主体はウマヅラハギと判断された。魚群の少ない北部域の佐田岬半島南側から速吸瀬戸にかけては，カタクチイワシと判断される小さな群れが分布していた。

9月の調査では豊後水道南部の芹崎から由良崎を結ぶ線の **St.** 11—14—23, **St.** 15—24, **St.** 15—16—25 に大きな群れが分布しているのが目立っている。又，速吸瀬戸南方の **St.** 62—63—64, **St.** 64—53—52 でも比較的大きな群れが多い。佐田岬南東沖の **St.** 54—65—66, **St.** 45—46 やその周辺域では小さな群れが分布している（**Fig.** 8）。大きな群れが分布している水域のなかで，水道南部のものはサバ類，ウルメイワシを主体とするもの，速吸瀬戸南部のものはウマヅラハギを主体とするものと判断された。小さな群れが分布するところではカタクチイワシと判断された。

群れ相互の水平距離は，3月には水道中央部付近で広く，佐田岬半島南側から速吸瀬戸周辺にわ



Fig. 8 Average size of fish-schools along interstation lines, 10—15 September 1972.

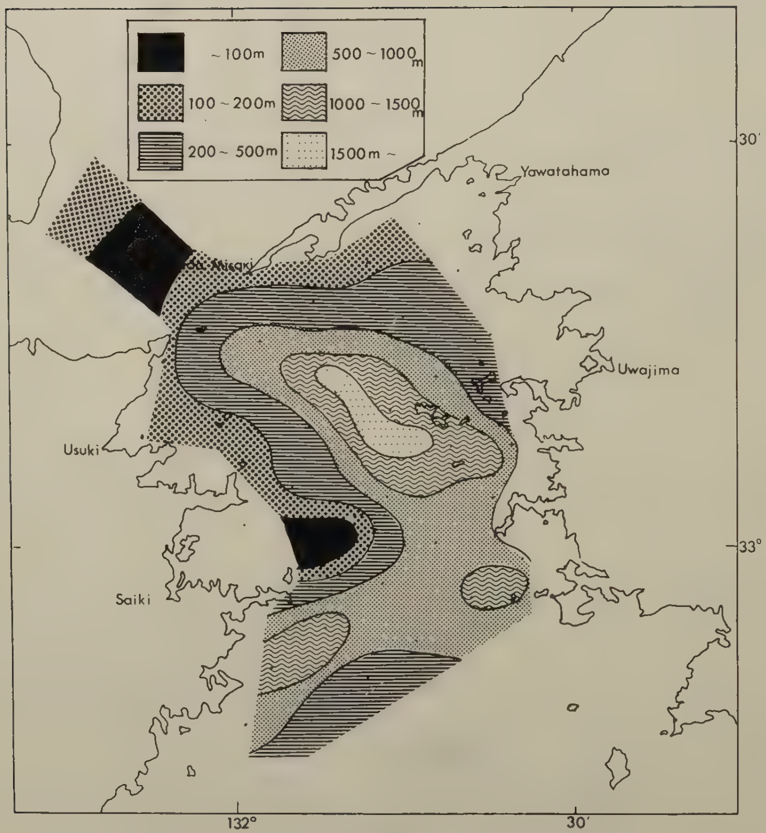


Fig. 9　Distance between fish-schools, March 1972.

たる水域と西部沿岸域で狭い（**Fig.** 9)。とくにカタクチイワシとみられる速吸瀬戸北側の群れと，ウマヅラハギとみられる鶴見崎沖の群れの間隔は100m以下であった。

9月には速吸瀬戸南側の一部のウマヅラハギとカタクチイワシとみられる群れ，佐伯湾口のウマヅラハギ主体の群れ，由良崎付近のカタクチイワシ，ウルメイワシ混合の群れ等が100—200mと比較的狭い間隔で分布し，水道中央部や南部で1,000—1,500mと最も広い間隔で分布しているものがみられる。中央部のものはウルメイワシ，カタクチイワシ等が混在する群れ，南部のものはサバ類，ウルメイワシ主体の群れである（**Fig.** 10)。3月に比べて9月における水道全域での群れの間隔は広狭の差が小さい。

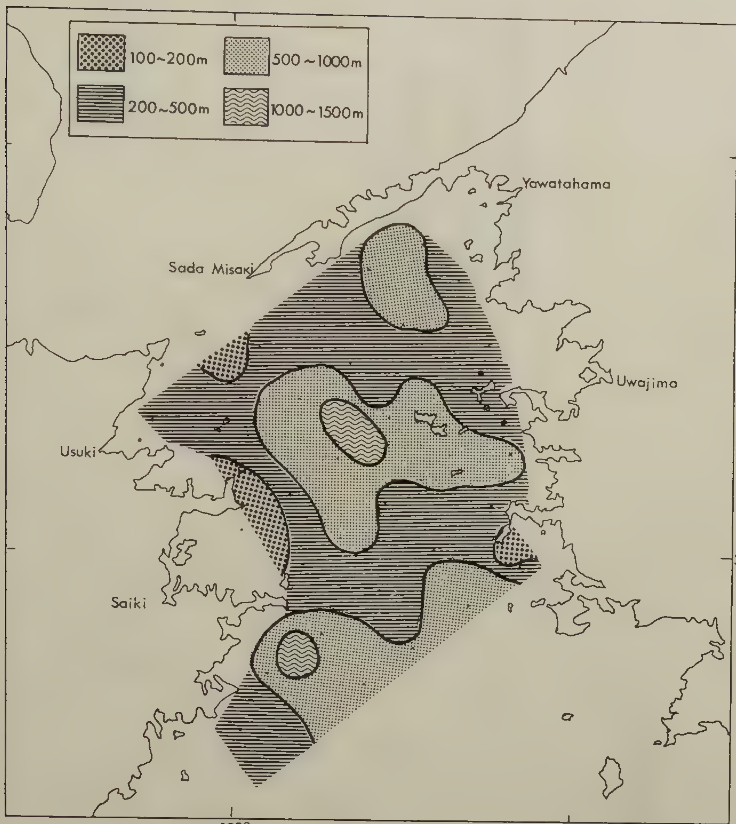


Fig. 10 Distance between fish-schools, September 1972.

4. 9月における潮流による魚群分布の変化

9月7—15日には，2点間の1時間毎連続調査又はブロック毎の同時調査を実施して，潮流と魚群分布との経時的変化を調べた。

9月7日に速吸瀬戸に近い St. 62—63 の間を9時から16時にかけて連続調査した。速吸瀬戸の潮流は9時10分の憩流から南流に移り，12時41分に4.1ノットの最強を示し，15時41分に憩流に戻った。この間1時間毎に出現した魚群を記録した。この魚群の大部分はカタクチイワシである。魚群量は9時10分の憩流をすぎた9時30分から10時の間に最大を示し，潮流が強くなるにつれてゆるやかに減少し，その最強時をすぎた13時30分から14時の間に急激に減少した（Fig. 11)。

9月10—13日，15日の5日間に調査した A, B, C, D, E の5ブロック毎に，日間差を無視して北流時から憩流時に至る間の魚群分布の変化を調べた。北流最強時前後における魚群量の分布を Fig. 12 に示す。魚群は鶴見崎南方の St. 11—12—13 間及び34‰等塩分線に沿った中，南部域，速吸瀬戸南部の St. 62—63—64—53 間，佐伯湾口域の St. 31—41—42間に多い。北流から憩流に達した頃の魚群量分布を Fig. 13 に示す。魚群は鶴見崎南方の St. 11—12 間，St. 13—14—23 間，

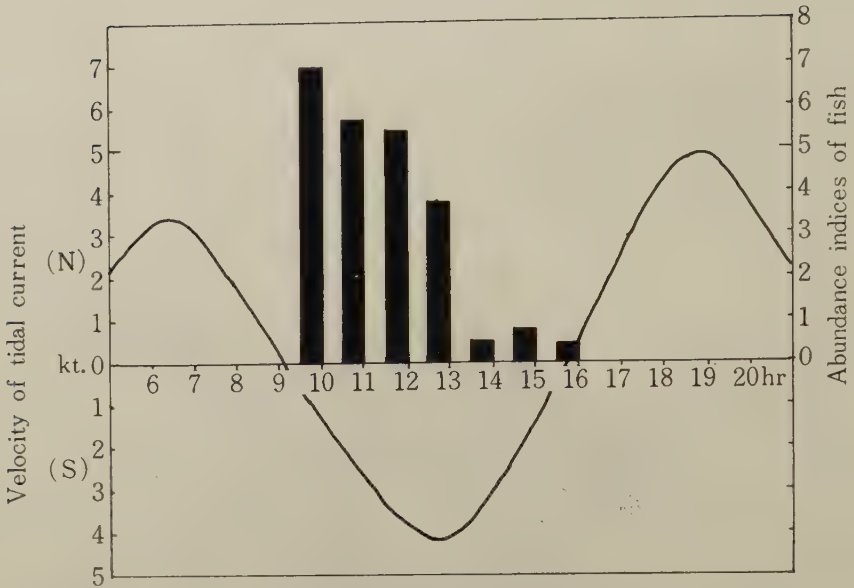


Fig. 11　Velocity of tidal current at HAYASUI-SETO (strait) and abundance index of fish between st. 62–63, 7 September 1972.　Curve denotes velocity of tidal current and bars denotes abundance indices of fish.

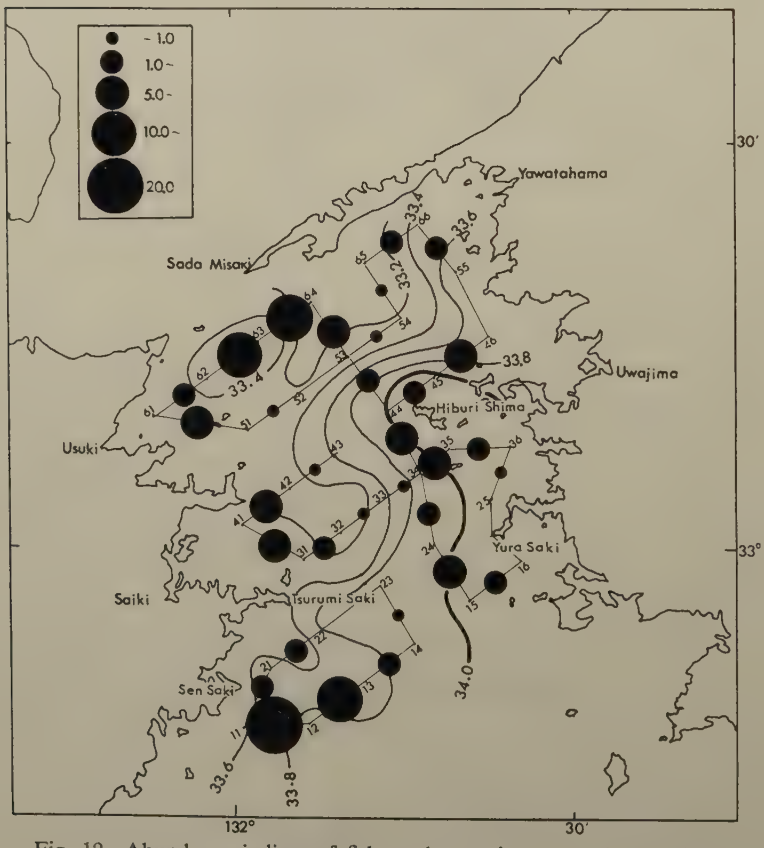


Fig. 12　Abundance indices of fish at the maximum northward tidal current, 10—15 September 1972.

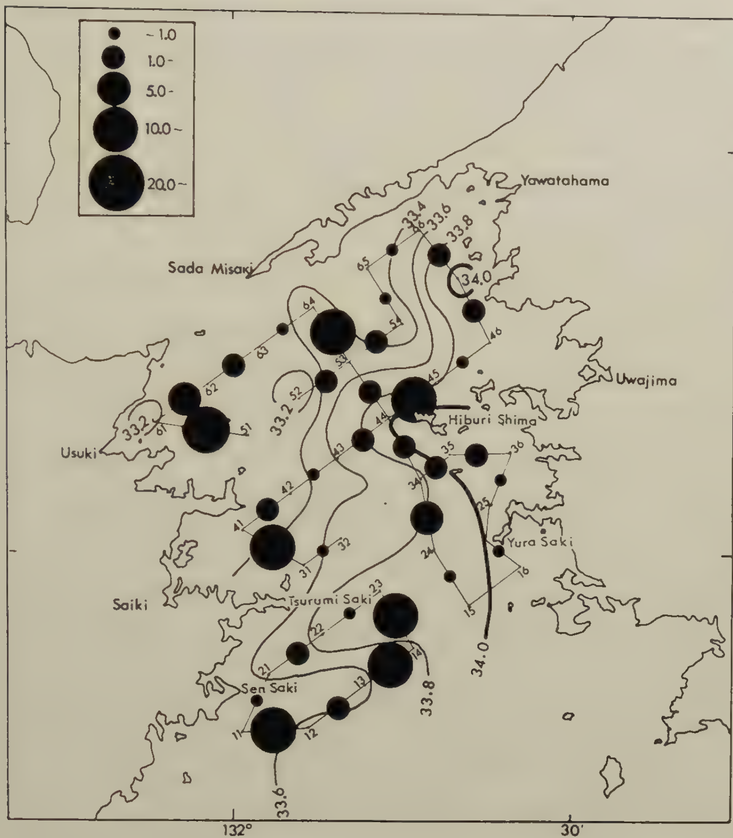


Fig. 13 Abundance indices of fish at the slack water, 10—15 September 1972.

日振島北の St. 44—45 間，その北の St. 53—64間，臼杵湾口の St. 51—61—62 間及び佐伯湾口の St. 31—41 間に多い。

考　　察

Fig. 3，**Fig.** 4 によって3月と9月における魚群分布量を比較すると，3月に比べて9月に多くしかも水道の北部にまで多く分布している。このことは豊後水道域における魚群が，初冬季から初春季にかけては外海域に移動するものが多いため減少し，夏季には外海域から来遊するため増加するという季節的な傾向を示していると考えられる。魚群の分布について神浦（1958，1966）は，日向灘のイワシ類は沿岸水帯と沖合水帯との境界付近や，水温傾度の大きい場所に多く集合する傾向が強いと報告している。今回の豊後水道における調査のなかで3月の魚群分布をみると，鶴見崎沖に多く集群しているのが目立っている。水道西部沿岸域では冬季を中心に内海系水の南下流が卓越するが，3月においてもこの勢力が強く鶴見崎付近が外洋系水との境界となり，ここに魚群が多く集ったものと考えられる（**Fig.** 3）。9月における魚群分布はほぼ水道全般にわたって多く，3月の

ように局部的に多く集群する傾向はみられない。このことは，9月は夏季を中心とした外海域からの来遊群がまだ水道域に滞留し，内海系水の流出も弱い時期であるのごとくに偏った分布域は形成されないものと考えられる（Fig. 4）。

魚群の鉛直分布は，3月の調査では鉛直混合の盛んな場所ほど深い傾向を示し，また水道北部よりも南部で深い層に分布している（Fig. 5）。9月の調査では水道南部の25℃以上の高温域には全く分布せず，24—25℃の間にも極めて少ない。全般に22—23℃の間に多く，この水温がこの時期における適温の範囲と考えられる。又，3月に比べて上下の分布の幅が広いが，これは群れの厚さが大きいサバ類，ウルメイワシ，及びウマヅラハギ等の魚群が多いためと考えられる（Fig. 6）。Fig. 4に示した9月における30m層塩分分布では，外洋水は水道南東部から差し込んでいると判断される。これに対して Fig. 6 に示した水温の鉛直分布では，水道中央部から外洋水が北上していることを示唆している。このように30m層の塩分の水平分布からみた外洋水の入り込みが，水温の鉛直分布にも示されるとは限らない。水塊の区別とそれに伴う魚群の影響について，温度，塩分を合せて再検討する必要がある。

個々の群れの大きさは，外洋系水の影響の強い水道南部域ほど大きい傾向がみられた（Fig. 7, Fig. 8)。群れの大きさは，神浦（1966）によると伊予灘，豊後水道，日向灘のカタクチイワシは，外海域の日向灘で最も大きいものが出現すると報告している。又，Fig. 3, Fig. 4 のなかで魚群量が多くないにもかかわらず Fig. 7, Fig. 8のなかで群れの大きなものが示されているところ，例えば水道南部の St. 13—14—23, St. 15—16—25 等では，出現した群れの数は非常に少なく定点間の魚群量としては小さいが，大型の群れが分布していることを示すものである。

各群れ間の距離の大小は魚群集合の度合を示すものである。Fig. 9, Fig. 10 に示したように，沿岸域ほど各群れ間の距離は小さく密集しており，外洋系水の影響の強い沖合ほど各群れ間の距離は大きく粗に分布している。

速吸瀬戸に近い St. 62—63 において，魚群は憩流時には多かったが，4ノット前後の強流になると急激に減少している（Fig. 11)。このことは，カタクチイワシのような小型の浮魚類では4ノット前後の強い流れには耐えられなくなり，移動してしまう可能性を示唆している。

摘　　要

1972年3月及び9月に魚群探知機を用いて，豊後水道域における魚群の分布及び潮流による影響について調査した。その結果を要約すると次の通りである。

魚群は，内海系水の南下が強い3月には内海系水と外洋系水との境界付近に多い。内海系水の南下が弱く，外海域から来遊した魚群が多く滞留している9月には，境界域等に局部的な集群をする傾向はみられない。鉛直分布をみると，鉛直混合の盛んな場所で深い傾向がみられる。9月には水温25℃以上のところには全く出現せず，22—23℃のところに多く分布する。

群れの大きさは，3月には内海系水と外洋系水との境界域で大きい。9月には外洋系水の影響が強い水道南部域で大きい。群れ間の距離の計測結果から，沿岸域では密に，沖合域では粗に分布す

る傾向がみられた。

潮流が魚群の分布に与える影響をみると，潮流の速い水道北部の速吸瀬戸付近で，憩流後南流が4ノット前後になると魚群は急激な減少を示した。

文　　献

浅見忠彦，1962：太平洋南区のカタクチイワシ *Engraulis japonica* (HOUTTUYN) に関する研究．南水研報，16，1―55.

花岡藤雄，1972：土佐湾及びその周辺海域における沿岸浮魚類の分布様式―Ⅲ，魚探による魚群調査からみた海域間の比較．昭和47年度日本水学会秋季大会発表資料．

海上保安庁，1967：豊後水道及付近潮流図第6213号．

海上保安庁，1972：昭和47年潮汐表第1巻．201―205.

科学技術庁，1973：豊後水道海域における精密海洋観測総合研究報告書．115―212.

科学技術庁，1975：豊後水道海域における精密海洋観測総合研究報告書．1―82.

神浦藤雄，1958：魚群探知機の記録よりみたイワシ類魚群の分布構造について，南水研報，9，76―83.

神浦藤雄，1966：魚探記録からみたカタクチ群の大きさと出現状況について，南水研報，23，31―42.

能津純治，1965：豊後水域におけるカタクチイワシ *Engraulis japonica* (HOUTTUYN) の漁業生物学的研究．大分水試調研報，3，1―137.

横田滝雄，1953：日向灘豊後水道のイワシ類の研究．南水研報，2，128―231.

南西水研報
Bull. Nansei Reg. Fish.
Res. Lab. No 15, 1983

On the Symbiotic Relationship between *Porphyra* Species and Attached Bacteria, and a Bacterial Pathogen in White Rot

Jun-ichi Tsukidate

Abstract

The thesis deals with the symbiotic relationship between *Porphyra* species and attached bacteria in conjunction with the white rot which has caused the most serious damage to the industry of *Porphyra* cultivation in Japan. This study made clear for the first time the fact that a bacterium identified as *Beneckea* sp. was one of pathogens in the white rot of *Porphyra*. The host-parasitic interaction in the outbreak of the white rot was revealed after pathological and physiological studies had been made on a bacterial pathogen, *Beneckea* sp. The following results were obtained in the present study and the basic knowledges were provided to foster the healthy lavers in the *Porphyra* farming, resulting in the increasing yields in its industry.

1) A bacterial flora was investigated on the healthy thalli of *Porphyra* grown both in laboratory and on the rocks or the nets in the sea. The composition of microflora was determined according to the scheme of Shewan *et al.* *Micrococcus*, *Achromobacter*, *Flavobacterium/Cytophaga* and *Pseudomonas* were isolated from the healthy *Porphyra* thalli *in vitro*. Of these, *Achromobacter*, *Flavobacterium/Cytophaga* and *Pseudomonas* were often isolated. *Micrococcus*, *Achromobacter*, *Flavobacterium/Cytophaga*, *Pseudomonas*, *Aeromonas* and yeast were isolated from the healthy *Porphyra* thalli grown on the rocks or the nets in the sea. Of these, *Achromobacter*, *Flavobacterium/Cytophaga* and *Pseudomonas* were often isolated. Therefore, it was concluded that *Achromobacter*, *Flavobacterium/Cytophaga* and *Pseudomonas* were dominant parasitic bacteria on *Porphyra* thalli.

2) An epidemiological survey of the pathogenic bacteria was periodically carried out with the *Porphyra* grown on the rocks or the nets in the sea in 1973, 1974 and 1975 fiscal year of the *Porphyra* cultivation. In any fiscal year, *Beneckea* sp. was isolated from *Porphyra* thalli whenever red and white cells increased in number on its thallus surface, resulting in the deterioration of lavers and the subsequent occurrence of the white rot.

3) In order to prove causality, healthy *Porphyra* thalli which were cultured *in vitro* were

Received: November 15 1982. Contribution No. 128 from the Nansei Regional Fisheries Research Laboratory, Ōno-chō, Hiroshima-ken, 739-04, Japan

suspended in the medium inoculated with *Beneckea* sp.. After bacterial inoculation, the host always deteriorated and caused the white rot. Beneckea sp. was reisolated from the above deteriolated thalli at a ratio of 95% and 100% in one and other four cases out of five experiments, respectively.

4) Investigation on the growth of *Beneckea* sp. and associated bacteria which were isolated from the host plants, *Porphyra* species, indicated that *Beneckea* sp. grew well at 20℃ and with some amino acids like aspartic acid and glutamic acid. In addition, it was found that there existed different nutritional requirement between *Beneckea* sp. and other parasitic bacteria.

5) From the view point of nutritional relationship between *Porphyra* and attached bacteria, extracellular products of *Porphyra* were analized and identified as organic matters and amino acids using labelled carbon and a Beckman 121 amino acid analyzer, respectively. It is the first time that amino acids are detected in the culture medium composed mostly of inorganic nutrients at the end of incubation of *Porphyra*.

6) It was indicated that the growth of bacteria attached to *Porphyra* thalli were stimulated by providing amino acids. The release of carbon dioxide was also ascertained in the culture medium of bacteria using labelling carbon. However, sorts of utilized amino acids and amount of released carbon dioxide were different among bacteria at generic level.

7) *Porphyra* was tested for its ability to grow in axenic culture when organic nutrients and carbon dioxide were provided. As a result, no growth of *Porphyra* was observed in culture medium containing any of the following substances: amino acids, vitamines, nucleic acid and plant hormons. On the contrary, carbon dioxide, in the presence of twice as much as those of artificial seawater used in the present study, supported the normal growth of *Porphyra*.

8) In summary, *Porphyra* grew well in the presence of carbon dioxide released by attached bacteria and amino acids as extracellular products of *Porphyra* stimulated the growth of bacteria. Above results indicate that the symbiotic relationship exists between *Porphyra* and attached bacteria. *Porphyra* became unhealthy when oceanographic and meteorological conditions change and may exudate amino acids which are readily available for *Beneckea* sp. It seems justifiable therefore that this nutrirional interaction leads to the vigorous growth of *Beneckea* sp., resulting in causing the white rot.

Contents

Chapter 1. General introduction

Porphyra tenera KJELLMAN is the seaweed most commonly used for food in Japan. *Porphyra* farming has been best accomplished by some of the most sophisticated techniques used in seaweed mariculture. Recently the annual crop has attained a level of 10 billion sheets of dried laver. However, *Porphyra* farming is influenced by meteorological and oceanographic conditions, and productivity is still far from constant. In addition, diseases have caused a deterioration in quality as well as low production. *Porphyra* is cultured in limited areas of protected bays, so microorganisms like diatoms, fungi and bacteria are more apt to attach to *Porphyra* than to seaweeds on exposed coasts. A problem in *Porphyra* farming is also pollution by industrial and domestic wastes.

Many researchers have attempted to solve the problems of disease in *Porphyra* farming by studies of epidemiology, physiology and bacteriolgy. Infectious and non-infectious diseases occur in both macrothallus and conchocelis stages. SUTO *et al.* (1972) gave a general review of diseases in *Porphyra* farming in Japan. According to them, red rot, chytrid blight and tumour were classified as infectious diseases, but bud blight, chill blight, diatom felt and white blight were regarded as non-infectious diseases.

Red rot is caused by *Pythium porphyrae* n. sp. (TAKAHASHI 1970, TAKAHASHI *et al.* 1970). The first symptom is a bluish green colour of the thallus. Finally a red ring appears around part of the bluish green area (ARASAKI, 1974, SASAKI and SATO, 1969). Data on the pathogen life cycle, host-parasite interaction, pathogenesis and epidemiology have been accumulated for red rot (ARASAKI 1947, SASAKI and SATO 1969). TSURUGA and NITTA (1960) reported a relationship between the pathogen, seawater temperature, salinity, and nutritional value of the thallus. ARASAKI (1962) took measures to prevent the spread of the disease according information on these relationships. Biochemical characteristics of the pathogen were investigated by ARASAKI *et al.* (1968), SASAKI and SAKURAI (1972), KATO *et al.* (1973a, b). FUJITA and ZENITANI (1976, 1977a, b, c). and FUJITA (1978).

The chytrid blight is caused by an unidentified Phycomycetes. The infected thalli become dark red. As the disease becomes serious, the colour of the plant changes to greenish white beginning at the upper end of the thallus. (Kumanoto Prefecture Laver Research Laboratory, 1969; Saga Prefecture Aquaculture Research Experimental Station (1969). ARASAKI (1960) and MIGITA (1969) investigated the morphology of the pathogen and the parasitic ecology in the host plant and showed the mechanism by which the pathogen invaded the cells. They attempted but failed to grow the pathogenic fungus in artificial media. ARASAKI *et al.* (1960) and MIGITA (1969) described the symptoms of the chytrid blight in infected *Porphyra* collected

by fishermem and the staffs of several prefectural fisheries experimental stations. The chytrid blight occurs widely in *Porphyra* farms infected by red rot.

The shot hole disease was first reported by SUTO and UMEBAYASHI (1954). Pinholes made by mechanical stimuli such as fine sands are apt to become as large as 1-4mm in diameter when the salinity is reduced. This disease is characterized by perforations in wellgrown thalli in November and December. It often occurs near river mouths when sands are discharged by flood waters right after a heavy rainfall. NOZAWA and NOZAWA (1955, 1957) succeeded in causing the shot hole disease experimentally. In farms where the salinty was reduced as much as 4-8%, they found disordered plants with the same symptoms as plants in their laboratory. Typical symptoms developed on plants growing at salinities of 6-8%. Symptoms of the shot hole disease were described by ARASAKI (1956) as follows. Small red spots spread over the thallus surface and become small holes afterwards; although the diseased thalli do not decay, their growth occasionally stops; when the salinity of the ambient sea becomes normal, the plants grow again; in advanced stages of the disease, parts of the plant become reddish purple and greenish, and groups of dead cells dot the thallus surface; ultimately holes form when the dead cells are lost.

Tumors are the result of abnormal cell divisions enhanced by deleterious chemical substances contained in industrial effluents. The symptoms of this disease are crape like upheavals induced by proliferating vegetative cells. The thalli damaged by tumors stop growing and become yellowish. The occurrence of tumors in a *Porphyra* farm was reported by FUJIYAMA (1957a, b) for the first time. He investigated the anatomy, cytology and physiology of the tumrs on *Porphyra tenera* and assumed that unknown microsubstances originating from the industrial effluents were responsible for the tumors. The substances causing tumors were studied by KAWABE and MIZUNO (1963), KAWABE *et al.* (1972), ISHIO *et al.* (1972), WATANABE and KATO (1972). KATO and WATANABE (1972) measured the amounts of DNA in the diseased cells, and KATO (1972) described the finestructure of these cells.

A bud blight develops often on thalli which are less than 1-2 cm long and believed to be physiological disorder. This disease occurs at farms which are offshore and sometimes also at the mouths of bays, and may spread extensively to the whole cultivation area. Smaller buds are more susceptible to the disease than larger buds, and even several celled germlings are often damaged. The symptoms of this disease are dullness and a reddish discoloration of a part of on the entire thallus, a distortion at the tip of the bud and a weakening of its base. The damaged plants are stunted or stop growing and finally wear away. Even when the plants heal they remain distorted and contain abnormal cells and chromatophores. The causes of this disease and the cultural manipulation needed to control it were studied by YAMAZAKI *et al.* (1956), AKASAKA (1956), TAMURA (1956) and ARASAKI (1956).

The chill blight is a physiological disorder occurring when thalli are exposed to cold wind for long times during ebb tides. Damaged parts of thalli become reddish brown at first and at more advanced stages they become polygonal or dendroid. Microscope examination shows that the cells are reddish violet in color and that the plastids shrink, resulting in discoloration. Finally the cells loose their contents and appear as large cells with vacuoles (AKASAKA *et al.* 1961).

when a diatom falt densely covers the thallus surfaces, *Porphyra* decays and stops growing (SUTO *et al.* 1972). OKAMURA (1909) reported that the diatoms were responsible for dense floras of bacteria living in the mucus substances secreted by attached species of *Synedra* and *Licmophora* on the surfaces of *Porphyra*. In fact, the existence of a mucus-like substance can be ascertained on rotted areas of *Porphyra* containing dead cells. *Synedra* grows rapidly in a low salinity, and *Licmophora* in a high salinity. The explosive increases of both diatoms have often caused serious demage to *Porphyra* farming. One example of the low yield resulting from attack by *Synedra* was reported from Korea long ago (Chosen Sotokufu Suishi 1934). Recently, the destructive damage caused by *Licmophora* was reported in the western part of the inland sea (Yamaguchi Prefectural Naikai Fishery Experimental station 1972). In the latter case, the blooming of *Licmophora* occurred three months earlier than usual (November and December) and was thought to be due to abnormal stagnant water. General speaking, a diatom felt is apt to develop on unhealthy thalli growing in unfavourable environments (SUTO *et al.* 1972).

The white blight is also a well known physiological disorder. The diseased thalli rot after they turn whitish or reddish. There are many references to white blight in early and advanced stages and the serious economic damages caused by the disease in various areas of Japan (Fukuoka Prefecture Ariake Fisheries Experimental Station 1968, Fisheries Agency 1967; Miyagi Prefecture Fisheries Experimental Station 1968, Kesenuma Branch of Miyagi Prefecture Fisheries Experimental Station 1968, Chiba Prefecture Naiwan Fisheries Experimental Station 1968). Pathological studies were made on *Porphyra* affected by this disease by WATANABE and KATO (1970), KATO *et al.* (1970) and KATO and WATANABE (1971). The initiation mechanism of white blight was studied by WATANABE *et al.* (1968) and YAMASHITA (1969).

Of all these diseases in *Porphyra* farming, red rot and white blight are of the greatest econmic importance and occur extensively somewhere in Japan every year, resulting in serious problems in the laver industry. Though the casual organism in red rot is known to be *Pythium porphyrae*, white blight is considered to be caused by several unusual environmental factors such as too little exposure to the air and lack of sunlight, and protective measures have not been fully established yet, though some attempts have been made to reduce the damage.

The present thesis constitutes a series of the author's studies of physio-nutrition (TSUKIDATE 1968, 1971a, 1974b), pathology and bacteriology (TSUKIDATE 1970a, c, 1971b, c, 1973a, b, 1974a, 1977b) and especially deals with the symbiotic relationship between *Porphyra* species and attached bacteria in conjunction with the white rot disease (TSUKIDATE 1977a).

Before going further, the author wishes to express his hearty thanks Dr. Tomitarô MASAKI, Professor of Hokkaido University, for critically reading and improving the manuscript. Also the author wishes to acknowledge his great indebtedness to Dr. Takashi KIMURA and Dr. Isamu Tsujino, Professors at Hokkaido University, during the Preparation of the manuscript. Further, the author offers his appreciation to Drs. Hiroshi YABU and Yoshio EZURA, Hokkaido University, for their very valuable advice. To Dr. Luigi PROVASOLI, the author's thanks are extended for suggesting this problem and for inspiration in his study. Also, thanks go to Dr. H. William JOHANSEN of Clark University, for correcting the English and making valuable comments. The author is glad to thank the following for offering him excellent facilities and for encouragement during the work: Dr. Shunzo SUTO, retired Professor of Tohoku University, Dr. Nobuhiko HANAMURA, former Director of the Nansei Regional Fisheries Research Laboratory, Dr. Yukimasa KUWATANI, Director of the Nansei Regional Fisheries Research laboratory, Dr. Yunosuke SAITO, former Chief of the Aquaculture Division of the Nansei Regional Fisheries Research Laboratory, Dr. Buhei ZENITANI, Professor of Nagasaki University, and Mr. Masaaki SASAJIMA of the Tokai Regional Fisheries Research Laboratory.

Chapter 2. History

The first report of white blight causing serious damage to the *Porphyra* industry in Japan was made under the name of "Hakufu-byo" by ARASAKI (1947) in which he described detailed symptoms on the basis of an epidemiological study on the Pacific coast of central Japan. According to him, the diseased thalli discolor to reddish or whitish from their apices downwards and ultimately detach from "Hibi", (floating nets for cultivation) as a result of shrinkage and deterioration leading to weakened holdfasts. In the cells the starlike chromatophores deform and the protoplasts discolor to violet or whitish. The white blight occurs from late November to late December and becoming serious especially when the water temperature remains high and rains persist. The disease develops mainly on well grown thalli in the lower parts of "Hibi", while small thalli in the upper parts are usually healthy.

The Miyagi prefecture Fisheries Experimental Station reported *Porphyra* with symptoms similar to "Hakufu-byo" or "Shirogusare-byo" (ARASAKI 1947, 1956) but differing in the season of its occurrence and the environmental factors inducing the disease. They also called it "Shirogusare-byo", and found that it occurred around the neap tide during October and

November. The meteorological conditions responsible for the disease are considered to be: (1) wind calm during low tide; (2) air temperature dropping below dew-point; (3) low air temperature and strong wind or (4) an increase of seawater pH under the environmental conditions mentioned in (1)to (3) (Fisheries Agency 1965).

Data on another type of "Shirogusare-byo" was given by Chiba Prefecture Naiwan Fisheries Experimental Station (1947, 1956) and the Research Department of the Fisheries Agency (1965, 1967). The disease starts to develop immediately after the elongation of *Porphyra in* November. In this case, the characteristic feature is the deterioration of buds composed of 2 or 3 cells growing on the "Hibi". The symptoms of damaged thalli are dullness, a discoloration to reddish brown, a decrease in thickness together with a reddish coloration when seen in transmitted light. Diseased cells can be also observed following: (1) the affected cells are grouped; the spaces between cells broaden; chromtophores are deformed; the cells are full of granules and the cell contents become whitish and indistinct; (2) the cells enlarge (3) large vacuoles as in malnourished cells and (4) the violet dead cells are in a line or in a patch.

The disease with the above symptoms occurred in Saga Prefecture in 1967, in Fukuoka Prefecture in 1967 and 1968 and in Kumamoto Preferture in 1969. This was reported in 1968 and 1970 by the Fisheries Experimental Stations belonging to the corresponding Prefecture.

From data provided by the fisheries research stations located around *Porphyra* farms, SUTO *et al.* (1972)checked carefully symptoms of the white blight and classified the disease into 2 groups. One of them is the physiological disorder which Arasaki described in 1947 and 1956 while the other disease was considered to be caused by bacteria. The latter disease has become called white rot and develops on laver irrespective of size and growing depth. To reduce damage of the white blight, fishermen can try to improve environmental factors by controling the levl of "Hibi", but the white rot has become serious in spite of such protective measures. This suggests that white rot is of bacterial origin.

So far two diseases in which the causual organisms are bacteria are known in *Porphyra* farming in Japan. One is Ôhan-byo, a disease of the conchocelis stage. This disease was reported by Nozawa and NOZAWA (1959), KATO (1967) and TANIGUCHI (1970, 1977a, b) but its detailed description has not yet been given. "Ryokuhan-byo", or green spot rot, is the other disease of the leafy stage. NAKAO *et al.* (1972) isolated *Pseudomonas* and *Vibrio* from diseased *Porphyra* and succeeded in causing green spot rot in healthy thalli by inoculation *in vitro*. FUJITA *et al.* (1972) made further bacteriological studies of *Porphyra* and isolated *Beneckea and Agarbacterium* from infected thalli in addition to the two bacteria mentioned above. After experiments *in vitro* these newly isolated bacteria were shown to be the organisms causing the red discoloration. TOMINAGA (1970) isolated bacteria from 9 species and 2 subspecies

belonging to 8 genera from infected *Porphyra* collected in 6 prefectures in Kyûshû and on the Pacific coast of Japan during 1970 and 1971, but could not find any pathogens among them. However, there have been no bacteriological studies of the white rot so far.

The existance of microflora on *Porphyra* has been known since Sieburth's study in 1976, but until quite recently no work has been done on relationships between host and attached bacteria for the purpose of reducing white rot in *Porphyra* farming.

On the contrary, there are several reports on the nutrient requirements of marine algae in axenic culture. PROVASOLI (1950) indicated that membraneous *Ulva lactuca* grown in axenic culture became filamentous. TATEWAKI and PROVASOLI (1963) found that 3 species of Antithamnion did not grow well in axenic culture but the addition of vitamin B_{12} accelerated their growth. FRIES (1963, 1966, 1970) and FRIES and PETTERSSON (1968) investigated the effect of some nutritious chemical elements on the growth of several species of red algae in axenic culture. TSUKIDATE (1970) stated that the membraneous plant of *Grinnellia americana* grew well in axenic culture using seawater from the mouth of Tokyo Bay, but became dendroid in axenic culture using seawater from the bottom of Tokyo Bay. However, the dendroid condition became membranous by the addition of bacteria isolated from other well grown membranous of same alga.

The above facts indicate that marine algae grow well only in the presence of bacteria attached to the host.

Chapter 3. Bacterial flora of healthy *Porphyra*

A high population of bacteria exists in the surface mucilage of *Porphyra* (SIEBURTH 1976). Therefore, it is very difficult to isolate epiphytic bacteria perfectly from the *Porphyra* thallus. However, an understanding of the bacterial flora associated with helthy thalli of *Porphyra* will help in searching for pathogens causing white rot. For this reason, this chapter deals with the bacteria isolated from wild, stocked and cultivated thalli of *Porphyra*.

Materials and methods

1) Algal materials

2 species of *Porphyra* were used for the experiments. Groups of 10 plants of *Porphyra leucosticta* Thuret were collected 6 times from February 17, 1971 to October 30, 1973 and cultured *in vitro*. Groups of 12 plants of *Porphyra* yezoensis Ueda growing on rocks and "Hibi", near the Nansei Regional Fisheries Research Laboratory, Ôno, Hiroshima Prefecture were collected 11 times from February 5, 1972, to February 8, 1978.

2) Bacteriological medium

Table 1. Composition of artificial sea water used.

NaCl	2.4 g
$MgSO_4$ $7H_2O$	0.8 g
KCl	70 mg
$CaCl_2$ $2H_2O$	55 mg
$NaHCO_3$	16.8 mg
$NaNO_3$	20 mg
Na_2 glycero PO_4	2.5 mg
Metal solution	1 mℓ
Pure water	100 mℓ
Metal solution 1 mℓ =	
H_3BO_3	0.2 mg
$FeCl_3$ $6H_2O$	0.048 mg
$MnCl_2$ $4H_2O$	0.144 mg
$CoCl_2$ $6H_2O$	0.04 mg
$ZnCl_2$	0.01 mg
Na_2EDTA	1 mg

Table 2. Composition of medium forisolation and preservation of bacteria.

Stock solution	100 mℓ
Meat extract	0.2 g
Peptone	0.5 g
Pota ssium nitrate	0.05 g
Agar	1.5 g
Stock solution 100 mℓ =	
NaCl	2.4 g
$MgSO_4$ $7H_2O$	0.8 g
KCl	70 mg
$CaCl_2$ $2H_2O$	55 mg
$NaHCO_3$	16.8 mg
$NaNO_3$	20 mg
Na_1glycero PO_4	2.5 mg
Metal solution	1 mℓ
Pure water	100 mℓ
Metal solution 1 mℓ =	
H_3BO_3	0.2 mg
$FeCl_3$ $6H_2O$	0.048 mg
$MnCl_2$ $4H_2O$	0.144 mg
$CoCl_2$ $6H_2O$	0.04 mg
$ZnCl_2$	0.01 mg
Na_2EDTA	1 mg

The medium used for isolation was modified from ZOBELL's medium (ZOBELL 1946) and was prepared using artificial seawater (Table 1). The artificial seawater employed was modified from a madium used by SUTO (1960). Sodium glycerophosphate was used instead of disodium phosphate to avoid precipitation after sterilization and coppeı was eliminated because a sufficient amount was supplied from sodium chloride and magnesium sulphate contained in the orginal artificial seawater.

3) Isolation and enumeration of bacteria

A sample of *Porphyra* was homogenized for 1 minute in a homogenizer cup with 10 ml of the sterilized artificial seawater at 3,000 r.p.m. Serial 10-fold dilutions were made of the homogenates with the sterilized artificlal seawater and a 0.05 ml or 0.1 ml homogenate taken from each dilution was spread on a plate of the isolation medium. The plates were incubated at 20℃ for 7 days. Colonies were counted with the naked eye and the bacterial populations were represented as the cell number per square centimeter. To obtain pure cultures, the whole colonies, which were estimated as one hundred in number, were picked up from the best plates.

4) Identification

Each isolate was identified to genus on the basis of cell morphology and biochemical tests according to SHEWAN's scheme (SHEWAN *et al.* 1960b).

5) Cell morphology

After each isolate was incubated at 20℃ for 1-7 days, cell size and shape, gram staining characteristics, flagellation and motility were investigated according to a routine method.

6) Biochemical tests

Oxidase tests were carried out according to Kovacs's method (KOVACS 1956) and carbohydrate utilization was tested according to the method used by LEIFSON (1963).

Besides the above tests, favorable temperatures for growth, VP test, MR test, indol production, hydrosulphide production, decrboxylation of aminoacids, hydrolysis of carbohydrate and nitrate reduction were studied.

Results

As shown in Table 3, the dominant genera of bacteria on the healthy thalli of *Porphyra* growing *in vitro* were *Achromobacter*, *Flavobacterium/Cytophaga* and *Pseudomonas* as well as small amounts of *Micrococcus*. On the other hand, the microorganisms associated with healthy thalli of *Porphyra* on rocks and "Hibi", mostly belonged to *Achromobacter*, *Flavobacterium/Cytophaga* and *Pseudomonas*, and small amounts of *Micrococcus*, *Aeromonas* and yeast (Table 4). *Aeromonas* was the only genus isolated from healthy thalli but not *in vitro* thalli. Yeast was found exclusively on thalli from the sea. *Flavobacterium/Cytophaga* seemed to attach the young thalli more often than the aged ones.

Table 3 Generic distribution of microorganisms isolated from the healthy thalli of *Porphyra* cultured in laboratory.

Sampling date	*Porphyra* thalli No.	*Micrococcus* %	*Achromobacter*	*Flavobacterium/ Cytophaga*	*Pseudomonas*	No. of the isolate	Length and width of *Porphyra* thalli
Feb. 17, '71	1		38		62	117	4 cm×6 cm
Mar. 11, '71	2	13	38	47	2	117	2 cm×3 cm
	3	16	36	43	5	100	2 cm×3 cm
	4	2	7	87	4	45	2 cm×3 cm
May 24, '71	5			75	25	12	1 cm×2 cm
Mar. 25, '72	6		65	17	18	99	0.5cm×6.5cm
Mar. 30, '73	7	16	35	53	1	140	0.1cm×1.5cm
Oct. 30, '73	8		11	78	11	93	2mm long
	9		38	54	8	122	3mm long
	10		29	42	29	149	0.1cm×1 cm

Table 4 Generic distribution of microorganis ms and yeast isolated from the healthy thalli of *Porphyra* growing on nets or the rocks in the sea.

Sampling date	*Porphyra* thalli No.	*Micrococcus*	*Achromobacter*	*Flavobacterium/ Cytophaga*	*Pseudomonas*	*Aeromonas*	Yeast	No. of the isolate	length and width of *Porphyra* thalli
Feb. 5, '72	1	%	71	16	6	3	4	115	0. 5cm × 0. 5cm
Feb. 8, '72	2		65	25	1	5	4	107	0. 5cm × 1 cm
Feb. 22, '72	3		26	9	17	5	43	135	1 cm × 1 cm
Mar. 3, '72	4		19	72	2		7	104	0. 5cm × 0. 5cm
Mar. 7, '72	5		17	78	2	1	2	108	1 cm × 2 cm
Dec. 4, '73	6		6	8	76		10	138	5mm long
Dec. 18, '73	7		9	47	43		1	116	1 cm × 1 cm
Oct. 30, '75	8		3	63	33		1	45	1. 5mm × 1. 5mm
Nov. 22, '75	9		33	28	39			36	1 cm × 1 cm
Nov. 25, '75	10		8	8	84			48	1. 5cm × 0. 5cm
Feb. 8, '78	11	1	10	65	24			111	2 cm × 2 cm
	12		14	65	21			186	2 cm × 2 cm

Discussion

As *Achromobacter*, *Flavobacterium/Cytophaga* and *Pseudomonas* were always detected in the epiphytic bacterial flora on *Porphyra* investigated in this study, it is likely that these 3 genera are dominant members of the bacterial flora on *Porphyra* thalli whether from the sea or the laboratory. *Aeromonas* was never isolated from the thalli grown *in vitro*, but was found on thalli grown in the sea. This might be due to repetitive inoculations to renew culture media *in vitro*. However, this is not yet clear. *Micrococcus* was present mainly on thalli grown *in vitro*, while yeast was isolated from those from the sea.

TOMINAGA (1973) investigated the bacterial flora of diseased thalli of *Porphyra* collected at farms all over Japan. He identified 9 species and 2 subspecies belonging to 8 genera, *Micrococcus lactis*, *M. roseus*, *Staphylococcus spidermidis*, *Achromobacter pestifer*, *A. parvulus*, *Flavobacterium fucatum*, *Agarbacterium uliginosum*, *Pseudomonas halestorga*, *P. membranoformis*, *Aeromonas hydrophila* subsp. *anaerognes*, *Serraeia macrescens* subsp. *Kiliensis*. In a study on epiphytic microorganisms isolated from *Polysiphonia*, *Ascophyllum* and the ambient sea, CHAN and MACMANAS (1969) reported the genera *Pseudomonas*, *Vibrio*, *Flavobacterium*, *Achromobacter* or *Alcaligenes*, *Escherichia*. *Staphylococcus*, *Sarcina* and a pink yeast *Rhodotorula*. MURCHELANO and BROWN (1969) indicated that bacteria isolated from *Monochrysis lutheri* and *Isochyrsis galbana*, planktonic algae growing in the laboratory, belonged to the genera *Pseudomonas*, *Flavobacterium*, *Achrombacter*, *Vibrio*, *Bacillus* and *Corynebacterium*.

BERLAND *et al.* (1969) also reported that epiphytic bacteria associated with 6 species of marine planktonic algae cultured in the laboratory belonged to *Pseudomonas*, *Flavobacterium*, *Achromobacter*, *Vibrio*, *Agarbacterium*, *Xanthomonas*, *Micrococcus* and *Staphylococcus*. KONG and CHAN (1979) isolated the genera *Pseudomonas*, *Vibrio*, *Alteromonas*, *Xanthomonas*, *Achromobacter*, *Flavobacterium* and *Micrococcus* from marine algae including 3 species of green algae, 2 species of brown algae and 2 species of red algae.

The papers cited above show that the major bacterial genera associated with marine algae are *Achromobacter*, *Flavobacterium* and *Pseudomonas*. Therefore it is assumed that these 3 genera are the most common epiphytic bacteria growing on the marine algae along the coast of Japan.

Chapter 4. Isolation and identification of pathogenic bacteria causing white rot.

In the course of an ecological study of *Porphyra* growing on "Hibi", in Nagai, Kanagawa Prefecture from 1966 to 1968, the author observed white blight. The disease did not occur every year even though meteorological and oceanographic conditions were quite similar and therefore the role of microocganisms in pathogenesis was considered. As shown in chapter 3, many bacteria thrive on healthy thalli of *Porphyra*. It is therefore indispensable that physiological and microbiological studies are made on infected *Porphyra* on the farms in order to understand pathogen and host-parasite interaction. For this purpose, the present chapter deals with a study from December, 1973 to Nobember, 1975 on variations in the bacterial floras on diseased thalli and the relationships between the disease and the presence of a bacterium which seems to be pathogenic.

Materials and methods

1) Algal materials

Porphyra yezoensis Ueda was collected from rocks in 1973 and 1974 and from "Hibi" near the laboratory in 1975. The alga were brought back to the laboratory immediately after collection and observed with the naked eye and under the microscope to check the viability and the degree of the disease.

2) Bacteriological media

In this chapter except a part of Identification and Biological tests was carried out according to the method described in chapter 2 unless stated otherwise.

3) Isolation and enumeration of bacteria

4) Identification

For identification of some material strains, the type strain of *Beneckea campbellii* ATCC-

25920 which had been been preserved as a stock culture at Laboratory of Bacteriology Faculty of Fisheries, Hokkaido University was employed. Other parts were carried out according to methods described in the preceding chapter.

5) Cell morphology

6) Biological tests

GC content in DNA, hydrolysis of chitin and resistance to salinity were tested on some bacterial strains according to BAUMANN *et al.* (1971a, 1973) and ALLEN *et al.* (1971) as well as SHEWAM *et al.* (1960b). DNA was extracted and refined by Marmur's method (cf. SAITO 1966) and the content of GC was expressed as mole percentage from DNA heat-denaturation curves. Other parts were carried out according to methods described in the preceding chapter.

Results

The number of bacterial strains isolated was 2950 in the culture season of 1973 and 2470 in the culture season of 1974 and 1980 in the culture season of 1975. Each bacterial isolate was identified to genus level and the composition of the microorganisms was shown as a percentage of the total number (Table 5). Most strains were assigned to *Achromobacter*, *Flavobacterium/Cytophaga*, *Pseudomonas* and yeast but a few strains could not be identified by the scheme of SHEWAN *et al.* (1960b). The unidentified strains were found at the 0.4% on the thalli of *Porphyra* collected on January 17, 1974, and abundant white and dead host cells were observed only on that day. These unidentified strains were 22.1% of the epiphytic bacterial flora on the healthy thalli of *Porphyra* but abundant white and dead host cells were not found find on the farm until 3 days later. The occurrence of the bacterial strains in question was as high as 20% and 30.7% on November 6 and November 13, 1975, respectively, but abundant white and dead host cells were detected only on thalli collected on November 10, 13 and 15 (Table 5). In other words, many white and dead host cells were detected up to the 4 days after the unidentified bacterial strains were isolated from the thalli.

The biochemical tests were made on the 3 unidentified strains assigned to *Beneckea* and *Beneckea campbellii* ATCC-25920 and the results (as well as their morphological characters) are shown in Tables 6 and 7.

Table 5. Generic distribution of microorganisms isolated from *Porphyra* thalli which were collected during December, 1973 and November, 1975.

Sampling date	*Achromobacter*	*Flavobacterium/ Cytophaga*	*Pseudomonas*	Unidentified	Yeast	No. of the isolate	Viable counts	Remarks of *Porphyra* cell
Dec. 4, '73	% 6.0	8.4	75.8	0	9.8	298	2×10^4/cm²	Healthy
Dec. 11, '73	0	53.0	27.7	0	19.3	393	6×10^3/cm²	Healthy
Dec. 18, '73	8.4	47.4	43.2	0	1.0	500	4×10^4/cm²	Healthy
Dec. 21, '73	0.8	16.4	82.8	0	0	244	2×10^4/cm²	Healthy
Dec. 25, '73	9.5	30.3	60.2	0	0	628	4×10^4/cm²	Healthy
Dec. 27, '73	0	18.3	71.1	0	10.6	142	1×10^4/cm²	Healthy
Jan. 5, '74	2.8	29.7	67.7	0	0	155	8×10^3/cm²	FC, GCC
Jan. 9, '74	7.4	15.7	69.4	0	7.5	134	8×10^3/cm²	FC
Jan. 17, '74	21.4	35.6	42.4	0.5	0	205	1×10^5/cm²	FC, WDC
Jan. 22, '74	25.2	14.7	55.4	0	14.7	251	1×10^4/cm²	FC
Nov. 22, '74	0	100	0	0	0	331	2×10^4/cm²	Healthy
Nov. 28, '74	89.3	10.7	0	0	0	373	2×10^4/cm²	Healthy
Dec. 5, '74	44.5	40.7	0	0	14.8	27	2×10^3/cm²	Healthy
Dec. 11, '74	36.4	63.6	0	0	0	132	2×10^4/cm²	Healthy
Dec. 19, '74	100	0	0	0	0	98	2×10^2/cm²	Healthy
Dec. 27, '74	75.0	25.0	0	0	0	144	8×10^2/cm²	Healthy
Jan. 6, '75	75.4	24.6	0	0	0	578	2×10^4/cm²	Healthy
Jan. 13, '75	58.8	20.6	20.6	0	0	126	2×10^3/cm²	Healthy
Jan. 18, '75	51.9	48.1	0	0	0	52	4×10^4/cm²	EV
Jan. 20, '74	11.5	8.6	52.0	22.1	5.8	173	1×10^4/cm²	Healthy
Jan. 23, '75	0	0	100	0	0	313	2×10^4/cm²	WDC
Jan. 28, '75	0	3.2	96.8	0	0	94	6×10^3/cm²	FC
Jan. 31, '75	34.5	10.3	34.5	0	20.7	29	2×10^3/cm²	Healthy
Oct. 30, '75	2.6	62.5	32.3	0	2.6	269	8×10^3/cm²	Healthy
Nov. 3, '75	0	41.0	59.0	0	0	100	4×10^3/cm²	Healthy
Nov. 6, '75	5.1	35.2	39.7	20.0	0	176	4×10^3/cm²	Healthy
Nov. 10, '75	2.6	67.9	29.5	0	0	190	8×10^3/cm²	WDC
Nov. 13, '75	20.6	26.3	22.4	30.7	0	281	1×10^4/cm²	WDC
Nov. 15, '75	24.9	75.1	0	0	0	217	2×10^5/cm²	WDC
Nov. 18, '75	40.4	31.2	28.4	0	0	109	4×10^4/cm²	Healthy
Nov. 22, '75	33.0	28.0	39.0	0	0	164	8×10^5/cm²	Healthy
Nov. 25, '75	8.3	8.4	83.3	0	0	174	4×10^5/cm²	Healthy

FC: faded chromotophores; GCC: groups of clumped cells;
WDC: white and dead cells; EV: enlarged vacuole.

Table 6. Morphological and biochemical characteristics of *Beneckea* isolated in 1973 and *Beneckea campbellii* ATCC-25920.

Characteristics	*Beneckea* isolated in 1973	*Beneckea* campbe ii ATCC-25920
Cells form	Moderate straight round	Moderate straight round
Gram stain	—	—
Motility	+	+
Flagella	Peritrichous	Peritrichous
Pigmentation	—	—
Kovacs oxidase	+	+
Catalase	+	+
Acid by (MOF) from		
Glucose	F	F
Maltose	+	+
Mannitol	+	+
Ribose	+	+
Sorbitol	+	+
Sucrose	+	[illegible]
Lactose	+	—
Arabinose	+	—
Fructose	+	+
Galactose	+	+
Xylose	+	—
Raffinose	+	+
Trehalose	+	+
Mannose	+	+
VP test	—	—
MR test	+	+
Indol	+	+
Hydro ysis		
Starch	+	+
Chitin	+	+
Gelatin	+	+
Casein	+	+
Tween 80	+	+
Alginate	—	—
Cellulose	—	—
Agar	[illegible]	—
Gelatin liquefaction	+	+
NO_3 reduction	+	+
NO_2 reduction	+	+
TMAO	—	+
Litmus milk	Peptonization	Peptonization
Decarboxylation		
Lysine	—	+
Ornithine	—	—
Arginine	—	—
Sensitivity to O/129	±	±
H_2S production	+	+
Phosphatase	+	+
Growth at 10% NaCl	+	—
Sea water require ment	H	H
Growth at		
0℃	—	—
5℃	—	—
30℃	+	+
37℃	+	+
40℃	+	—
Gluconate oxidation	—	—
NH_3 from peptone	+	+
Citrate	+	+
GC moles %	44. 2	46. 4

Table 7. Morphological and biochemical characteristics of *Beneckea* sp. isolated in 1974 and 1975.

Characteristics	Iso ated bacteria: *Beneckea* sp. isolated in 1974 38[a]	*Beneckea*sp. iso ated in 1975 35
Cells form	Moderate straight round	Moderate straight round
Gram stain	−	−
Motility	+	+
Flagella	Peritrichous 35[b]	Peritrichous 33
Pigmentation	−	−
Kovacs oxidase	+	+
Catalase	+	+
Acid by (MOF) from		
Glucose	F	F
Maltose	+	+
Xylose	+	+
VP test	−	−
MR test	+	+
Indol	+	+
Hydrolysis		
Starch	+	+
Chitin	+	+
Gelatin	+	+
Casein	+	+
Tween 80	+	+
Alginate	−	−
NO_3 reduction	+	+
Decarboxylation		
Lysine	−	−
Ornithine	−	−
Arginine	−	−
Sensitivity to O/129	+	+
H_2S production	+	+
Growth at 10% NaCl	+	+
Sea water requirement	H	H
Growth at		
0℃	−	−
5℃	−	−
15℃	+	+
20℃	+	+
25℃	+	+
37℃	+	+
40℃	+	+
GC moles %	45. 9+1. 1	45. 7+0. 7

a : Number of strains used; b: Number of strains having peritrichous flagella;
+ : Positive; − : Negative

Discussion

The unidentified bacterial isolate was assigned to *Beneckea* establishod by BAUMANN *et al.* (1971a), because of the morphological and biochemical following properties; gram negative, positive motility, peritrichous on solid media, monotrichous in liquid media, positive to oxidase, requirement of NaCl for growth, fermentation in glucose without gas production, hydrolysis of chitin and GC content in DNA in the range of 45 to 48% (Table 6). On the other hand, 3 strains of the genus *Beneckea* isolated in January, 1974, and January and November, 1975, had in common the properties shown in Tables 5 and 6, and seemed to belong to the same taxon.

7 species belonging to *Beneckea* were originally described by BAUMANN *et al.* (1971a) but *Beneckea* sp. inquestion differed from them in properties enumerated below.

Beneckea sp. differs from *B. parahaemolytica* in the utilization of carbohydrates including sucrose and xylose, from *B. neptuna* and *B. nereida* in the decarboxylation of arginine and growth at 40°C, from *B. alginolytica* in the utilization of xylose, from *B. pelagia* in flagellation, utilization of xylose and growth at 40°C and from *B. netriegens* in the utilization of xylose.

The properties examined in the present study were not enough to compare with the more detailed description by BAUMANN *et al.* (1971a). A further comparison was made with the type strains of 7 species in *Beneckea* and 1 species of *Vibrio*, all of which were treated as marine *Vibrio* by LEE *et al.* (1978). *Beneckea* sp. isolated in the present study was different from 4 type strains, Beneckea *parahaemolytica* ATCC-17802, *B. neptuna* ATCC-25919, *B. campbellii* ATCC-25920 and *B. alginolytica* ATCC-17749, in the utilization of the carbohydrates cellulose, lactose, raffinose, arabinose, sucrose sorbitol and xylose, as well as in the TMAO test, growth at 10% NaCl and growth at 15° and 37°C. It was also different from *B. pelagica* ATCC-25916 in the utilization of the carbohydrates lactose, mannose, raffinose, sorbitol and xylose, and in the TMAO test and growth at 15° and 37°C, from B. natriegens ATCC-14048 in the utilization of the carbohydrates cellulose, lactose, mannose, sorbitol and xylose, in the catalase test, TMAO test and indol production. It differed from *B. nerida* ATCC-25917 in the utilizaiton of the carbohydrates lactose, mannitol, mannose, sorbitol and xylose, and in the catalase test and TMAO test. It differed from *Vibrio fisheri* in the utilization of the carbohydrates cellulose, lactose, mannitol, mannose, raffinose, sorbitol and xylose, and in the TMAO test, MR test, indol production, growth at 10% NaCl and growth at 30°C and 37°C. *Beneckea* sp. was compared to *B. campbelli* as described by BAUMANN *et al.* (1973) and, more precisely, with *B. campbellii* ATCC-25920 preserved in the Labortory of Bacteriology. The properties of both species included hydrolys of starch, chitin, gelatin, casein, tween 80 and alginate, decarboxylation of lysine, ornithine and arginine, test oxidase, catalase, VP test, MR test, indol production and growth at temperatures between 0° to 37°C. They differed in the utilization

of carbohydrates including sucrose, arabinose and xylose, decarboxylation of lysine, growth at 10% NaCl and growth at 40°C.

Beneckea sp. could not be assigned to any known species and appears to be hitherto unreported although more studies are necessary.

The relationships of white rot to environmental factors, such as the differences between atmospheric and seawater temperatures and desiccation at low tide, will be discussed below.

According to KATADA (1967), *Porphyra* utilizes large amounts of nutrients, especially when the seawater temperature drops gradually at the beginning of the growing season. He speculated that a shortage of dissolved nutrients such as nitrogen, phosphorus and carbonic acid for normal metabolism. On the other hand, fishermen say that *Porphyra* is more apt to be infected with the disease when the differences between atmospheric and seawater tempratures are big during the low tides at dawn. FUJIKAWA (1937) found that growth was optimal when exposed to air for 2-4 hours per day.

The author checked exactly the days corresponding with 4 hours or more exposure at dawn, the occurrence of abundant white and dead cells of *Porphyra* and a difference of at least 8°C between atmospheric and seawater temperatures (Table 8, Fig. 1). During the experimental time only 4 combined these features. A discrepancy of 9.8°C was found on January 12, 1974, 5 days before the occurrence of white rot, 9.5°C on January 13. 1975, 7 days before a serious outbreak of the disease and 9.5°C on November 4, 1975, 2 or 9 days before the presence of many white and dead host cells. In other words, *Beneckea* sp. was found in the *Porphyra* farm on the 2nd to 9th days after difference of 8°C or more between atmospheric and eawater stemperatures was deteced.

Moreover, the symptom of the disease observed in the present study was identical to that described as the white rot by SUTO *et al.* (1972) (Plates 1,2).

From the data mentioned above, it is suspected that *Beneckea* sp. is one of pathogens of white rot.

Table 8. The occurrence of discrepancy more than 8°C in atmospheric and sea water temperatures when *Porphyra* tha i expose to the air more than 4 hours per day in 1974 and 1975.

Date	Temperature difference
Jan. 7, 1974	9.1℃
Jan. 12, 1974	9.8℃
Jan. 19, 1974	9.0℃
Jan. 20, 1974	7.9℃
Jan. 27, 1974	10.1℃
Jan. 12, 1975	8.9℃
Jan. 13, 1975	9.5℃
Jan. 28, 1975	4.0℃
Jan. 29, 1975	9.0℃
Jan. 31, 1975	9.3℃
Nov. 4, 1975	8.4℃
Nov. 22, 1975	9.2℃
Nov. 23, 1975	9.4℃

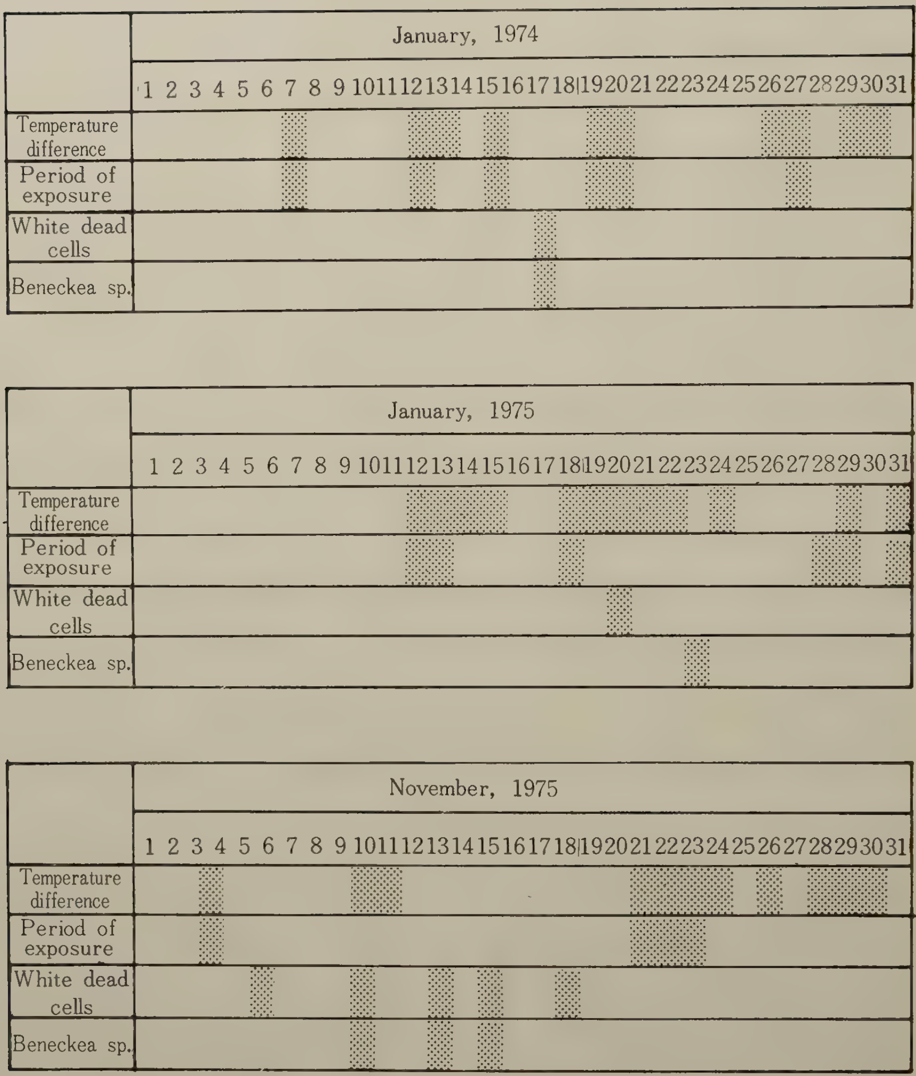


Fig. 1 The relation of maximum difference between atmospheric and seawater temperature, the period of exposure, the appearance of *Beneckea* sp. and white dead cells.

Chapter 5. Infection experiment of white rot

As mentioned in chapter 4, *Beneckea* sp. is considered one of pathogens causing white rot. In order to better understand the pathogenicity, *Beneckea* sp. was inoculated into *Porphyra*.

Materials and methods

1) Bacterial materials

Beneckea sp. was isolated on January 17 1974, from *Porphyra yezoensis* growing on inshore

rocks near the Nansei Regional Fisheries Research Laboratory, Ôno, Hiroshima Prefecture. Other bacteria including *Achromobacter* sp., *Flavobacterium/ytophaga* group and *Pseudomonas* sp. had been also isolated from *Porphyra leucosticta* on February 14, 1970, and all were incubated as a stock culture until the initiation of the experiment.

2) Algal materials

Porphyra yezoensis and *P. leucosticta* had been cultured in a 500 ml flask with aeration in artificial seawater at a light intensity of 3,000 lux and 17°C for the bud stage and at 10°C when a length of 1-3cm or more had been attained. The cultures were not axenic but the thalli of *Porphyra* were rinsed several times with the streilized artificial seawater before use.

3) Inoculation

Bacteria ranging from 10^3 to 10^4 per strain were suspended in 125ml Erlenmyer flasks containing 50ml of liquid medium (cf. Table 1) after being incubated for 1-5 days. 2 or 3 pieces of *Porphyra* were infected with bacteria by culturing for 1-2 weeks at 17°C and at 3,000 lux without aeration. Pathological observations on the thalli were made several times during the infectivity experiment.

4) Isolation and identification of bacteria from the infected *Porphyra*

Bacteria were isolated again from the infected *Porphyra* and were identified according to the methods described in chapter 3.

Results

The infectivity experiments were made 5 times using 4 species of bacteria isolated.

Experiment 1 (Table 9)

Thalli of *Porphyra* 1mm long were infected with *Beneckea* sp. and 4 days later bacteria were isolated from the infected thalli. The bacterial flora on uninfected *Porphyra* used as a control consisted of *Achromobacter* sp. (44%), *Flavobacterium/Cytophaga* group (16%) and *Pseudomonas* sp. (40%). Cells of infected *Porphyra* were all white and dead and only *Beneckea* sp. was isolated.

Table 9. Sign of white rot-like deterioration of *Porphyra* thalli caused artificially by bacteria.

Inoculated bacteria	Cell condition	Reisolated bacteria and their relative composition
None	Hea thy	*Achromobacter* sp. 44% *Flavobacterium/Cytophaga* group 16% *Pseudomonas* sp. 40%
Beneckea sp.	All are white and collapsing dead cells	*Beneckea* sp. 100%

Observation was made on the fourth day after inoculation of bacteria. The length of *Porphyra* thalli used in this experiment was 1 mm.

Experiment 2 (Table 10)

Thalli of *Porphyra* 0.2-0.3mm long were infected with *Beneckea* sp. and 8 days later bacteria were isolated from the plants. Infected *Porphyra* discolored to whitish and deteriorated. Only *Beneckea* sp. was found on infected *Porphyra*, whereas *Porphyra* used as control was very healthy.

Table 10. Sign of white rot-like deterioration of *Porphyra* thalli caused artificially by bacteria.

Inoculated bacteria	Cell condition	Reisolated bacterium and their relative composition
None	Healthy	
Beneckea sp.	All are white and collapsing dead cells	*Beneckea* sp. 100%

Observation was made on the eighth day after inoculation of bacteria. The length of *Porphyra* thalli used in this experiment was 0.2-0.3 mm.

Experiment 3 (Table 11)

Thalli of *Porphyra* 2mm long were infected with *Beneckea* sp. and 3 days later bacteria were isolated from the thalli. Cells of infected *Porphyra* were red or white, and moribund or dead. The *Porphyra* used as a control bore *Achromobacter* sp. (57%), *Flavobacterium/Cytophaga* group (12%) and *Pseudomonas* sp. (31%) whereas infected *Porphyra* had only *Beneckea* sp.

Table 11. Sign of white rot-like deterioration of *Porphyra* thalli caused artificially by bacteria.

Inoculated bacteria	Cell condition	Reisolated bacteria and their relative composition
None	Healthy	*Achromobacter* sp. 57% *Flayobacterium/Cytophaga* group 12% *Pseudomonas* sp. 31%
Beneckea sp.	Cells with a large vacuole Red or white Collapsing cells	*Beneckea* sp. 100%

Observation was made on the third day after inoculation of bacteria. The length of *Porphyra* thalli used in this experiment was 2 mm.

Experiment 4 (Table 12)

Thalli of *Porphyra* 1cm long were inoculated with *Achromobacter* sp. *Pseudomonas* sp. and *Beneckea* sp. and bacteria were isolated 6 days later.

The thalli infected with *Achromobacter* sp. were healthy and the bacteria isolated were *Achromobacter* sp. (60%), *Flavobacterium/Cytophaga* group (13%) and *Pseudomonas* sp. (27%). The thalli infected with *Pseudomonas* sp. were not healthy and most host cells were red or white, and moribund or dead. The bacteria isolated were *Micrococcus* sp. (6%), *Achromo-*

bacter sp. (4%) and *Pseudomonas* sp. (90%). The thalli infected with *Beneckea* sp. were whitish and the host cells were red of white, and moribund or dead. The bactecial flora on infected thalli consisted of *Micrococcus* sp. (4%), *Achromobacter* sp. (1%) and *Beneckea* sp. (95%).

Table 12. Sign of white rot-like deterioration of *Porphyra* thalli caused artificially by bacteria.

Inoculated bacteria	Cell condition	Reisolated bacteria and their relative composition
Achromobacter sp.	Healthy but cells	*Achromobacter* sp. 60% *Flavobacterium/Cytophaga* group 13% *Pseudomonas* sp. 27%
Pseudomonas sp.	Red or white collapsing cells	*Micrococcus* sp. 6% *Achromobacter* sp. 4% *Pseudomonas* sp. 90%
Beneckea sp.	Red or white collapsing cells	*Micrococcus* sp. 4% *Achromobacter* sp. 1% *Beneckea* sp. 95%

Observation was made on the sixth day afterinoculation of bacteria. The length of *Porphyra* thalli used in this experiment was 1 cm.

Experiment 5 (Table 13)

Thalli of *Porphyra* 1cm long were inoculated with *Achromobacter* sp., *Flavobacterium/Cytophaga* group, *Pseudomonas* sp. and *Beneckea* sp. and bacteria were isolated from the infected thalli 3 days later.

Table 13. Sign of white rot-like deterioration of *Porphyra* thalli caused artificially by bacteria.

Inoculated bacteria	Cell condition	Reisolated bacteria and their relative composition
Achromobacter sp.	Healthy	*Achromobacter* sp. 100%
Flavobacterium/Cytophaga group	Healthy	*Achromobacter* sp. 44% *Flavobacterium/Cytophaga* group 56%
Pseudomonas sp.	Healthy	*Achromobacter* sp. 40% *Pseudomonas* sp. 60%
Beneckea sp.	White and dead cells	*Beneckea* sp. 100%

Observation was made on the third day after inoculation of bacteria. The length of *Porphyra* thalli used in this experiment was 1 cm.

The thalli inoculated with *Achromobacter* sp. were healthy and only *Achromobacter* sp. was found on the hosts. The thalli infected with *Flavobacterium/Cytophaga* group were also healthy but isolated were *Achromobacter* sp. (44%) and *Flavobacterium/Cytophaga* group (56%). The thalli infected with *Pseudomonas* sp. were healthy and the bacteria consisted of *Achromobacter* sp. (40%) and *Pseudomonas* sp. (60%). The thalli infected with *Beneckea*

sp. deteriorated and host cells were red or white, and moribund or dead. No other bacteria except *Beneckea* sp. were isolated from the infected *Porphyra*.

Discussion

5 repeated experiments in which *Porphyra* were infected with *Beneckea* sp. revealed that the plants deteriorated lost their original color within 3 days, and died. *Porphyra* infected with *Beneckea* sp. was characterized by the sole occurrence of *Beneckea* sp. in 4 experiments, but including also *Micrococcus* sp. (4%) and *Achromobacter* sp. (1%)in a 5th experiment. The symptoms of the disease induced by *Beneckea* sp. were identical with those of the white rot described by Suto *et al.* (1972). There still remains some doubt about the bacterial medium being suitable for the growth of *Porphyra*. However, *Porphyra* used as a control grew well in the medium in question.

When inoculated with *Achromobacter* sp. or *Flavobacterium/Cytophaga*, *Porphyra* grew healthily, while *Porphyra* infected with *Pseudomonas* sp. grew well or lost the original cell color, which became red and white. When infected with *Pseudomonas* sp. that species occurred as much as 90% of the bacteria on the diseased *Porphyra*, but it occurred only 60% when the thalli were healthy. The reasons for the differences in bacterial populations was not clear.

When *Achromobacter* sp. was inoculated, 2 kinds of bacterial populations were present on *Porphyra*. One had a predominance of *Achromobacter* sp. and the other contained *Flavobacterium/Cytophaga* and *Pseudomonas* sp. in addition to *Achromobacter* sp. There may have been antagonistic relationships among these bacteria.

Chapter 6. Influence of nutrients, temperature and sodium chloride on bacterial growth

It is well known that bacteria isolated from marine algae utilize amino acids, organic acids, carbohydrates and other organic compounds for their growth (BERLAND *et al.* 1970). This study was carried out to learn the effects of amino acids, carbohydrates, temperature and sodium chloride on the growth of bacteria isolated from *Porphyra*.

Materials and methods

1) Bacterial materials

Micrococcus: 22 strains were isolated on February 22, 1980 from *Porphyra yezoensis* grown *in vitro*.

Achromobacter: 49 strains were isolated on February 22, 1980 from P. yezoensis grown *in vitro* and 26 strains on February 14, 1980 from *P. yezoensis* growing on inshore rocks near

the Nansei Regional Fisheries Research Laboratory, Ôno, Hiroshima Prefecture.

Flavobcterium/Cytophaga : 74 strains were isolated on February 22, 1980 from *P. yezoensis* grown *in vitro* and 121 strains on February 14, 1980 from *P. yezoensis* growing on inshore rocks near the Nansei Regional Fisheries Research Laboratory.

Pseudomonas : 43 strains were isolated on February 22, 1980, from *P. yezoensis* grown *in vitro* and 39 strains on February 14, 1980, from *P. yezoensis* growing on inshore rocks near the Nansei Regional Fisheries Research Laboratory.

Beneckea sp.: 1 strain was isolated on January 17,1974 from *P. yezoensis* growing on inshore rocks near the Nansei Regional Fisheries Research Laboratory and 6 strains on November 6, 1975 from *P. yezcensis* growing on "Hibi" near the Nansei Regional Fisheries Research Laboratory.

2) Bacterial media and culture conditions.

In the experiment on nutritional requirements, the amino acids used were alanine, valine, arginine, lysine, aspartic acid, glutamic acid, cystine, methionine, leucine, isoleucine, serine and phenylalanine; The carbohydrates tested were glucose, maltose, manitol, sucrose, lactose, arabinose and xylose. The incubations were kept under aerobic condition at 20°C for a week. For responses to temperature, incubation was at aerobic conditions for a week using the basic medium (Table 14), and 8 different temperatures: 0°, 5°, 15°, 20°, 25°, 30°, 37°, and 40°C. The sodium chloride tolerance experiments were carried out according to methods described by HIDAKA and SAKAI (1968), but the incubations were kept at 20°C for a week. Bacterial strains, ranging from 10^3 to 10^4/ml, were used for the experiments on the 2nd and 5th day after inoculation to a fresh medium, except for the experiment of sodium chloride tolerance, where a 6fold dilution of the basal medium (Table 15), containing 0.05% polypeptone and 0.01% yeast extract, was used for the culture before the initiation of the experiment. Growth was measured by counting the number of colonies or observing the density with the naked eye.

Table 14. Composition of basal medium used.

Stock solution (75%)		150 mℓ
Yeast extract		0.1 g
Polypeptone		0.5 g
Agar		1.5 g
Stock solution	100mℓ =	
NaCl	3 g	
KCl	0.07 g	
$MgSO_4\ 7H_2O$	0.26 g	
$MgCl_2\ 6H_2O$	0.5 g	
$CuSO_4\ 2H_2O$	0.1 g	

Table 15. Composition of Herbst's artificial sea water.

NaCl	3 g
KCl	0.07 g
$MgSO_4\ 7H_2O$	0.26 g
$MgCl_2\ 6H_2O$	0.5 g
$CaSO_4\ 2H_2O$	0.1 g
Pure water	100 mℓ

Results

A. Amino acid requirements

The results obtained are shown in Tables 16 and 17.

1) The strains of *Micrococcus* sp. grew well in a medium with glutamic acid, alanine and arginine.

2) Most strains of *Achromobacter* sp. grew well by adding alanine, valine and cystine. The strains originating from the laboratory utilized well glutamic acid, while it was not

Table 16. Influence of amino acids on growth of bacteria.

Bacteria / Amino acids	*Micrococcus* 22[a]	*Achromobacter* 49	*Flavobacterium/ Cytophaga* 74	*Pseudomonas* 43
Alanine	20[b]	42	40	+
Valine	–	45	34	40
Arginine	17	23	31	+
Lysine	–	28	29	23
Aspartic acid	–	25	30	+
Glutamic acid	+	40	+	+
Cystine	–	41	35	37
Methionine	–	24	35	17

+ : Growth; – : No growth; a: Number of strains used;
b: Numbers indicate number of positive strains.

Observations were made on the seventh day after incubation of bacteria which were isolated from *Porphyra leucosticta* cultured in laboratory.

Table 17. Influence of amino acids on growth of bacteria.

Bacteria / Amino acids	*Achromobacter* 26[a]	*Falvobacterium/ Cytophaga* 121	*Pseudomonas* 39	*Beneckea* 7
Alanine	+	65	+	6
Valine	+	56	36	5
Arginine	13[b]	59	38	–
Lysine	14	63	18	6
Aspartic acid	11	66	35	+
Glutamic acid	8	119	+	+
Cystine	+	55	15	4
Methionine	14	63	15	5
Leucine	12	–	–	–
Isoleucine	12	–	–	–
Serine	–	9	–	–
Phenylalanine	–	8	–	–

+ : Growth; – : No growth; a: Number of strains used.
b: Numbers indicate number of positive strains;

Observations were made on the seventh day after incubation of bacteria which were isolated from *Porphyra yezoensis* grown on nets or the rocks in the sea.

certain if the strains originating from the sea utilized this amino acid or not.

3) All strains of *Flavobacterium/Cytophaga* grew well using only glutamic acid.

4) Most strains of *Pseudomonas* also grew well using alanine, valine, arginine, lysine, aspartic acid, glutamic acid and cystine.

5) The growth of most strains of *Beneckea* sp. was good in supplemental alanine, valine, lysine, cystine and methionine, but glutamic and aspartic acid resulted in the good growth of all strains, of *Beneckea* sp.

B. Carbohydrate requirements

The results obtained are shown in Tables 18 and 19.

1) Most strains of *Micrococcus*, *Achromobacter*, *Flavobacterium/Cytophaga* and *Pseudomonas* grew well in the presence of glucose, maltose and manitol, but failed to grow with

Table 18. Influence of carbohydrates on growth of bacteria.

Bacteria / Carbohydrates	*Micrococcus* 22[a]	*Achromobacter* 49	*Flavobacterium/ Cytophaga* 74	*Pseudomonas* 43
Glucose	+	+	+	+
Maltose	+	46	73	+
Manitol	+	+	70	+
Sucrose	15[b]	40	45	34
Lactose	14	25	40	30
Arabinose	11	39	48	31
Xylose	—	5	41	39

+ : Growth; — : No growth; a : Number of strains used;
b : Numbers indicate number of positive strains.

Observations were made on the seventh day after incubation of bacteria which were isolated from *Porphyra leucosticta* cultured in laboratory.

Table 19. Influence of carbohydrates on growth of bacteria.

Bacteria / Carbohydrates	*Achromobacter* 26[a]	*Flavobacterium/ Cytophaga* 121	*Pseudomonas* 39	*Beneckea* 7
Glucose	+	+	+	+
Maltose	+	+	+	+
Manitol	+	120	+	+
Sucrose	20[b]	57	25	+
Lactose	14	56	21	+
Arabinose	21	65	20	+
Xylose	2	56	29	+

+ : Growth; — : No growth; a : Number of strains used;
b : Numbers indicate number of positive strains,

Observations were made on the seventh day after incubation of bacteria which were isolated from *Porphyra yezoensis* grown on nets or the rocks in the sea.

the addition of sucrose, lactose, arabinose and xylose.

2) The growth of all strains of *Beneckea* sp. was supported by all carbohydrates examined.

C. The effects of temperature

The results obtained are shown in Tables 20 and 21.

All bacterial strains used in the experiment failed to grow at 0° and 5°C, but grew at temperatures higher than 20°C except *Pseudomonas* where growth did not occur at 37°C and 40°C.

Table 20. Growth of bacteria at various temperatures.

Bacteria / Temperatures	*Micrococcus* 22[a]	*Achromobacter* 49	*Flavobacterium/Cytophaga* 74	*Pseudomonas* 43
0℃	−	−	−	−
5℃	−	−	−	−
15℃	+	+	+	+
20℃	+	+	+	+
25℃	+	+	+	+
30℃	+	+	+	+
37℃	+	+	+	−
40℃	+	+	−	−

+ : Growth ; − : No growth ; a : Number of strains used.

Observations were made on the seventh day after incubation of bacteria which were isolated from *Porphyra leucosticta* cultured in laboratory.

Table 21. Growth of bacteria at various temperatures.

Bacteria / Temperatures	*Achromobacter* 26[a]	*Flavobacterium/Cytophaga* 121	*Pseudomonas* 39	*Beneckea* 7
0℃	−	−	−	−
5℃	−	−	−	−
15℃	+	+	+	+
20℃	+	+	+	+
25℃	+	+	+	+
30℃	+	+	+	+
37℃	+	+	−	+
40℃				+

+ : Growth ; − : No growth ; a : Number of strains used.

Observations were made on the seventh day after incubation of bacteria which were isolated from *Porphyra yezoensis* grown on nets or the rocks in the sea.

D. Sodium chloride tolerance

The results obtained are shown in Tables 22 and 23.

1) The strains of *Micrococcus* and *Flavobacterium/Cytophaga* grew well in redistitilled

water containing 0.05% polypeptone and 0.01 yeast extract and was considered to be of terrestrial origin.

2) All strains of *Achromobacter* and *Pseudomonas* were marine bacteria which required monovalent and divalent ions, as their growth was observed only in Herbst's artificial seawater (c f. Table 15) used as a basal medium.

3) All strains of *Beneckea* sp. grew well at 2 concentrations of sodium chloride as well as in Herbst's artificial seawater and are, therefore, halophilic.

Table 22. Influence of sodium chloride and sea water on growth of bacteria.

Salts \ Bacteria	*Micrococcus* 22[a]	*Achromobacter* 49	*Flavobacterium/ Cytophaga* 74	*Pseudomonas* 43
DW[c]	+	−	49	−
0.5% NaCl	+	−	71	−
3% NaCl	3[b]	−	36	−
75% ASW[d]	+	+	+	+

+ : Growth; − : No growth; a : Number of strains used;
b : Numbers indicate number of positive strains; c : Distilled water; d : Artificial sea water.
Observations were made on the seventh day after incubation of bacteria which were isolated from *Porphyra leucosticta* cultured in laboratory.

Table 23. Influence of sodium chloride and sea water on growth of bacteria.

Salts \ Bacteria	*Achromobacter* 26[a]	*Flavobacterium/ Cytophaga* 121	*Pseudomonas* 39	*Beneckea* 7
DW[c]	−	62[b]	−	−
0.5% NaCl	−	116	−	+
3% NaCl	−	56	−	+
75% ASW[d]	+	+	+	+

+ : Growth; − : No growth; a : Number of strains used;
b : Numbers indicate number of positive strains; c : Distilled water; d : Artificial sea water.
Observations were made on the seventh day after incubation of bacteria which were isolated from *Porphyra yezoensis* grown on nets or the rocks in the sea.

Discussion

Beneckea sp. which was considered to be one of pathogens causing white rot, grew well in media containing alanine, asparatic acid and glutamic acid. On the other hand, 3 predominant genera of bacteria isolated from healthy *Porphyra*, *Achromobacter*, *Flavobacterium/Cytophaga* and *Pseudomonas* required all amino acids employed in the experiment for their growth.

All bacteria, especially strains of *Beneckea* sp. grew well at 20°C or more.

Marine bacteria were indicated as utilizing metabolites released by phytoplankton (ITURRIAGA and HOPPE 1977). WILLIAM *et al.* (1977) also showed the utilization of amino acids in seawater by marine bacteria. KONG and CHAN (1979) tested the nutrient requirements of bacteria isclated from several algae and found that the isolates utilized alanine, arginine and lysine among 119 organic compounds tested. CHAN and MCMANUS (1969) also reported that 22 out of 25 strains of bacteria isolated from 2 species of red algae required amino acids for their growth. These data are not suitable for comparison with the results from the present study, as organisms employed are different, but it seems likely that nutrients utilized by bacteria are different among bacterial taxa.

Chapter 7. Carbon dioxide produced by epiphytic bacteria

ALLEN (1971) postulated that epiphytic bacteria released carbon dioxide, as a result of his research on the relationships between macrophytes and bacteria in lakes.

In chapter 6, it was shown that bacteria associated with *Porphyra* required several kinds of acids for growth. Therefore it is reasonable to assume that bacteria decompose amino acids, leading to the production of carbon dioxide. The present experiment was carried out to study the release of carbon dioxide by bacteria associated with *Porphyra* after decomposing amino acids.

Materials and methods

1) Bacterial materials

1 strain of *Micrococcus* sp., 2 strains of *Flavobacterium/Cytophaga* group and 2 strains of *Pseudomonas* sp. were isolated on December 4, 1976 from *Porphyra yezoensis* on rocks near the Nansei Regional Fisheries Research Laboratory, located at Ôno, Hiroshima Prefecture; 1 strain of *Achromobacter* sp. on November 24, 1970 from *P. yezoensis* growing on "Hibi", near the Nansei Regional Fisheries Research Laboratory; 1 strain of *Beneckea* sp. on January 17, 1974 from *P. yezcensis* growing on rocks near the Nansei Regional Fisheries Research Laboratory.

2) Measurement of carbon dioxide released by attached bacteria

For measuring cabon dioxide released by attached bacteria, a special 50ml Frlenmeyer flask (YAMADA *et al.*, 1979) with a small built-in chamber was used (Fig. 2). A small amount of 1 μCi amino acid with labelled carbon (Table 24) was placed around the small chamber and mixed with 2ml of natural seawater and 1ml of bacterial suspension containing from 19^7 to

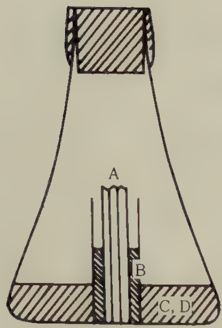


Fig. 2. Apparatus for producing and trapping ^{14}C.

10^8 cells. Inside the chamber, 1ml of methanol in which hydroxide hyamine 10-x (P. (diisobutylcresoxyethoxyethy l) dimethylbenzylammonium hydroxide) was dissolved to give a concentration of 1 M was placed with one 3×3cm sheet of accordion-folded filter paper (Toyo No. 2). After stoppering tightly (rubber stopper) and covering with a black cloth. each flask was placed on a shaker for 1 hour at 25°C, and then 1 ml of 6N HCl was added by injection outside the chamber. The amount of labelled amino acid used was calculated from specific gravity. The radioactivity of hydroxide hyamine and filter paper was measured using a Packard 3255 TRI-CARB liquid scintilation spectrometer. The amount of carbon dioxide released by the bacteria was expressed as a percent of carbon produced to the carbon supplied at the initiation of the experiment.

Table 24. Specific activity of labelled substrates in stock solution and quantities added in the experiments.

Amino acids	Specific activity mCi / m mol	Quantities μg
Aspartic acid	220	0. 55
Threonine	232	0. 51
Serine	162	0. 59
Glutamic acid	270	0. 59
Proline	290	0. 4
Glycine	112	0. 63
Alanine	164	0. 51
Valine	280	0. 4
Isoleucine	330	0. 38
Leucine	330	0. 43
Tyrosine	486	0. 35
Phenylalanine	486	0. 29
Histidine	348	0. 44
Lysine	330	0. 59
Arginine	324	0. 68

Results

The results are shown in Tables 25 and 26. Owing to the impurity of the labelled amino acids and to the complexity of the procedure carbon dioxide was produced up to 0.1% even if the experiment did not run. Therefore, the amount of carbon dioxide was considered to

Table 25. Amount of CO_2 produced by bacteria from various amino acids contained in media.

Bacteria / Amino acids	*Micrococcus* sp.	*Flavobacterium/ Cytophaga* group	*Pseudomonas* sp.	*Pseudomonas* sp.
Aspartic acid	3.1%	1.8%	13.5%	3.6%
Threonine	0	18.9	4.5	25.2
Serine	0.2	54.0	0	45.0
Glutamic acid	1.8	1.8	4.5	2.5
Proline	0.5	8.1	85.6	0
Glycine	0.1	0.2	2.3	45.0
Alanine	2.7	3.5	4.5	2.3
Valine	0.4	1.8	19.5	1.8
Isoleucine	0.8	22.5	1.8	1.7
Leucine	5.4	25.2	1.0	10.0
Tyrosine	1.0	7.2	0	9.5
Phenylalanine	7.6	1.6	0	5.4
Histidine	0	1.3	5.9	1.4
Lysine	0	4.9	0.4	6.8
Arginine	0	21.6	2.6	14.9

All bacteria were isolated on Dec. 4, 1976 from *Porphyra yezoensis* which grow on rocks in the sea. The amount of carbon dioxide is expressed as a percentage of carbon in the derived carbon dioxide to the amount of carbon supplied to the substrate.

Table 26. Amount of CO_2 produced by bacteria from various amino acids contained in media.

Bacteria / Amino acids	*Achromobacter* sp.	*Flavobacterium/ Cytophaga* group	*Beneckea* sp.
Aspartic acid	5.9%		21.8%
Threonine	0	15.4%	7.8
Serine	1.6	42.0	16.8
Glutamic acid	33.5	0	35.7
Proline	80.2	24.7	48.5
Alanine	0.9	0	29.7
Valine	1.4		0.5
Leucine	1.1	33.9	5.4
Tyrosine	1.4	0	0.5
Phenylalanine	1.7	0	0.5
Histidine	2.4	35.0	0.6
Lysine	0	24.4	3.3
Arginine	3.4	0	4.5

Achromobacter was isolated on Nov. 24, 1970 from *Porphyra yezoensis* which grow on nets in the sea.
Flavobacterium/Cytophaga was isolated on Jan. 20, 1975 from *P. yezoensis* which grow on rocks in the sea.
Beneckea was isolated on Jan. 17, 1974 from *P. yezoensis* which grow on rocks in the sea.
The amount of carbon dioxide is expressed as a percentage of carbon in the derived carbon dioxied to the amount of carbon supplied to the substrate.

be O when it was less than 0.1%. All bacterial strains used decomposed amino acids and produced carbon dioxide, although the amounts differed among the bacteria.

Micrococcus sp. decomposed several amino acids leading to the production of small amounts of carbon dioxide, but threonine, serine, glycine, histidine, lysine and arginine did not lead carbon dioxide production. *Flavobacterium/Cytophaga* group decomposed serine, leucine and threonine, resulting in the production of much carbon dioxide, but low amounts occurred when glutamic acid, alanine and phenylalanine were used. Different amounts of carbon dioxide was produced when histidine, lysine, arginine and isoleucine were decomposed by the above bacteria. *Pseudomonas* sp. showed high percentages of carbon dioxide from many amino acids, but the amounts often differed markedly between the 2 strains. *Beneckea* sp. produced much carbon dioxide by decomposing proline. glutamic acid aspartic acid, alanine and serine, but the production was extremely low when valine, tyrosine, phenylalanine and histidine were used.

Discussion

It became clear that *Beneckea* sp. decomposed aspartic acid and glutamic acid, resulting in a high production of carbon dioxide. This fact seems closely ralated to the increased growth of *Beneckea* sp. when these 2 amino acids were added to the media (chapter 6). It is therefore conceivable that aspartic and glutamic acids are important nutrients for *Beneckea* sp. 5 amino acids, serine, leucine, threonine, arvinine and lysine, are considered also important for growth of *Flavobacterium/Cytophaga* group which are predominant on healthy *Porphyra*; these bacteria utilized these amino acids, though the amounts of carbon dioxide produced differed among the bacterial strains. Proline was well utilized by nearly all the bacterial strains and carbon dioxide produced was as high as 80.2% in *Achromobacter* sp. 48.5% in *Beneckea* sp., 85.6% in *Pseudomonas* sp. and 24.7% in *Flavobacterium/Cytophaga* group. On the other hand, *Pseudomonas* sp. and *Achromo*monas sp. decomposed proline and produced the highest amounts of carbon dioxide of all amino acids tested, whereas the *Flavobacterium/Cytophaga* group produced the highest amount of carbon dioxide after decomposing serine. The numbers of amino acids highly decomposed were largest in *Beneckea* sp., and smallest in *Micrococcus* sp.

Chapter 8. Organic substances liberated by *Porphyra* as extracellular Products

It is well known that algae release many kinds of metabolites as extracellular product (FOGG 1962, LEFEVRE 1964, HELLEBUST 1974). Above all, the liberation of organic substances has been reported in many algae, eg., several marine algae (SIEBURTH 1969, MOEBUS and

JOHNSON 1974), 1 species belonging to the Chrysophceae (AARONSON 1971) and several marine phytoplankton (BERMAN and HOLM-HANSEN 1974). Little is known of organic substances as extracellular products in *Porphyra*. In chapter 6, bacteria associated with *Porphyra* were shown to utilize glucose and amino acids for their growth. It is probable that these organic substances originate from the host. The present study was carried out to show that *Porphyra* liberates organic substances.

Materials and methods

1) Algal material

Porphyra yezoensis was kept at $-20°C$ until the initiation of the experiment. The experiments started after culturing *Porphyra* for 1 week and the thalli were punched to make round pieces, 8mm in diameter.

2) Measurements of organic carbon liberated by *Porphyra*

The instrument used in the experiment was the same as described in chapter 7 unless otherwise noted. A small amount of 1 μCi or 10 μCi of sodium bicarbonate with labelled carbon was placed outside a small chamber in a flask and then mixed with 10 or 50ml of artificial seawater and 5 or 7 round pieces of *Porphyra*. The artificial seawater used was modified from the medium shown in Table 1 to deplete the organic substance by adding disodium phosphate instead of sodium glycerophosphate. The specific activity of carbon-14 in bicarbonate was 57.7 mCi/m mol. Hydroxide hyamine 10-x dissolved in methanol and filter papers (see chapter 7) were placed in the small chamber. 1ml of 12N HCl was used to stop the reaction leading to the production of carbon dioxide by lowing the pH to 2 or

Table 27 List of experimental condition in the production of organic carbon.

Experiment No.	Media ml	Quantities of carbon-14 added μCi	Hyamine 10-x ml	No. of pieces of *Porphyra yezoensis* used
1	10	10	3	7
2	10	10	3	7
3	10	10	3	5
4	10	10	3	7
5	10	10	3	5
6	10	10	3	5
7	10	10	3	5
8	10	1	1	5
9	10	1	1	5
10	50	10	3	5
11	50	10	3	5
12	50	10	3	5
13	50	10	3	5

less. Measurements were made after shaking for another 1 hour to trap thoroughly the carbon dioxide produced outside a small chamber to filter papers. The organic compounds produced from the labelled inorganic carbon during photosynthesis remained in the medium outside the small chamber; the amounts were determined by measuring the radioactivity of the medium. The scintilator used was an instagel made by Packard. The radioactivity of carbon-14 absorbed by pieces of *Porphyra* was measured using an Aloka GM counter. The experimental conditions are shown in Table 27.

Results

Results obtained are shown in Table 28. The amounts of extracellular products released by *Porphyra* varied depending on plant viability, concentration of carbonate in medium, temperature and light intensity. The final values of the radioactivity of organic carbon in the medium ranged from 0.6 to 4.1% of the initial value of carbon dioxide supplied.

Table 28. Radioactivity of organic carbon found in the media at the end of the experiments.

Experiment No.	Radioactivity at the beginning μCi	Radioactivity at the end	
		dpm	μCi
1	10	434, 416	0. 195
2	10	318, 190	0. 143
3	10	271, 413	0. 122
4	10	596, 279	0. 268
5	10	136, 172	0. 061
6	10	741, 141	0. 333
7	10	407, 634	0. 183
8	1	76, 911	0. 034
9	1	45, 751	0. 020
10	10	536, 232	0. 241
11	10	691, 581	0. 311
12	10	874, 732	0. 394
13	10	919, 476	0. 414

dpm : Disruption per minutes.

Discussion

It was elucidated that *Porphyra* released organic matter produced during photosynthesis.

MOEBUS and JOHNSON (1974) reported that *Ascophyllum nodosum* discharged dissolved organic matter. AARONOSON (1971) indicated that *Ochromonas danica* released carbohydrates, nucleic acid, proteins and lipids. BERMAN and HOLM-HANSEN (1974) showed that marine plankton liberated photoassimilated carbon as dissolved organic substance into eutrophic water in amounts of 6-12% and into oligotrophic waters 17-27% of photoassimilation. They also found that

marine phytoplankton released orgnic substance only 5% of photoassimilation in logarithmic growth phase. TANAKA *et al.* (1974) demonstrated that the amounts of extracellular metabolic exuded by phytoplankton in Lake Biwa amounted to 1-11% of the total carbon of photosynthesis. They detected considerable quantities of glycollic acid in the water and suggested that this chemical was liberated by phytoplankton and might play an important role as nurishment for microorganisms.

It has been known that considerable amounts of free amino acids and peptides are liberated by planktonic algae (HELLEBUST 1974). FOGG and WESTLAKE (1955) showed that these nitrogenous substances accounted for as much as 59.6% of the total extracellular organic matter in *Chlamydomonas* sp. and 60.9% in *Anabaena* sp.. SIEBURTH (1969) detected carbon, carbohydrate, protein and polyphenol substances in metabolites discharged by several marine algae, and found that the amounts of total organic carbon liberated ranged from 54.2mg C/100g/h in *Ascophyllum nodosum* to 4.4mg C/100g/h in *Chondrus crispus*.

Table 29. Radioactivity caliculated in one piece[a] of *Porphyra yezoensis* at the end of the experiments.

Experiment No.	Radioactivity at the end cpm
2	2, 532
	2, 133
	2, 898
	2, 295
4	2, 360
	3, 029
	1, 860
8	218
	200
	156
	198
	168
	204
13	1, 628
	1, 618
	1, 442
	1, 160

cpm : Count per minutes ; a : 50. 24mm^2.

As stated before, the minimum final value of radioactivity of organic carbon in the medium was 0.6% of the initial value of carbon dioxide supplied. This value can be changed to 0.0145 μg on the basis of specific activity. This calculated value corresponds to 0.88mg C of organic carbon extracellularly liberated for 1 hour by *Porphyra*, on the basis of 100g dry weight. From this transformed value, the final value of organic carbon in the present experiment ranged from 0.88mg C/100 g/h to 5.88mg C/100 g/h. The values obtained are smaller than those described by SIEBURTH (1969), but the seaweed taxa are different. On the other hand, the values, of ^{14}C-assimilation of *Porphyra* (Table 29) are mostly identical to data which TSUKIDATE (1968) showed using healthy *Porphyra* and the amounts of organic carbon liberated in the present experiment are therefore considered reasonable.

Chapter 9. Exudation of amino acids into the environment by *Porphra*

As descibed in Chapter 8, *Porphyra* releases organic carbon as much as 0.6-4.1% of the carbon supplied as inorganic form. Some unicellular algae discharge organic carbon in amino acids, as has been shown by FOGG and WESTLAKE (1955), FOGG (1962), HELLEBUST (1974) and JOLLEY and JONES (1977). Therefore, in the present study the media in which *Porphyra* grew were analysed for the existence of extracellular amino acids.

Materials and methods

1) Algal material

Porphyra tenera used was collected in Matsukawa-ura, Miyagi pref. 10 healthy plants 2-5cm long were grown at 3,000 lux at 10°C for 23 days, or at 20°C for 8 days in 500ml Erlenmeyer flasks in the modified artificial seawater described in the Materials and Methods of Chapter 8. At the end of the time in culture, the thalli were somewhat paler and more mature, but no white and dead cells were present. Most thalli attained a length of 10cm and a width of 1cm.

2) Amino acid analyse

After removing *Porphyra*, the media were filtered successively through 0.45μm and 0.22μm milipore filter to eliminate microoorganisms. The filtered samples were evaporated in a water bath kept at about 40°C until reduced to 200ml or one tenth, the original volume. The concentrates were passed through an ion exchange resin of Umberlite CG-120 Type 3 in a colum 1.5cm in diameter and 20cm in length after washing the resin with 30-40ml of distilled water and injecting 0.01N HCl until pH reaches 2. Each loaded column was eluted with about 100ml-200ml of a 2N ammonia solution. The fluates were completely evaporated under vacuum at about 40°C. Before passing through the resin, the concentrates were sometimes evaporated thoroughly and dissolved with 5ml of 1N HCl in 45ml of methanol.

The solutions were filtered through Toyo No. 2 filter paper and then passed through the resin. Recovery of amino acids with this preparation was 82-80% except for 40% of the cystin.

Table 30 Composition of buffer used.

Sodium citrate $2H_2O$	19.6 g
Conc. HCl	16.5 mℓ
Thiodiglycol 25% solution	20 mℓ
Caprylic acid	0.1 mℓ
Final volume	1,000 mℓ

3) Measurements of amino acids

Samples were calculated to a fixed amount with 30% formic acid and again evaporated. In each sample, a buffer solution (Table 30) of 0.1 ml was added

prior to analysis. A beckman 121 type amino acid analyser was used.

Results

As described in Chapter 6, bacteria attached to *Porphyra* utilized amino acids for their growth. Therefore, free amino acids analysed in the prerent study seem to be a surplus of the acids exuded by *Porphyra* over the consumption of bacteria. The amount and composition of the free amino acids liberated by *Porphyra* into the media are shown in Table 31 and Figs. 3, 4 and 5. Many amino acids were discharged into the media by *Porphyra* during the experiment. When *Porphyra* was cultured at 10°C for 23 days, amino acids produced extracellulary by 973.8mg dry weight of the plant contained the highest amount

Table 31. Amount and composition of free amino acid which *Porphyra thalli* liberated in culture medium.

Age of culture	23 days	8 days
Temperatures	10℃	20℃
Amino acids	μg / 973. 8mg on dry basis	μg / 316. 7mg on dry basis
Aspartic acid	319. 2	168. 5
Threonine	166. 6	43. 6
Serine	735. 0	185. 5
Glutamic acid	102. 9	53. 9
Proline	333. 5	0
Glycine	360. 0	180. 0
Alanine	204. 7	68. 2
Valine	105. 3	23. 4
Methionine	14. 9	0
Isoleucine	52. 4	17. 5
Leucine	64. 5	30. 1
Tyrosine	162. 9	72. 4
Phenylalanine	115. 5	82. 5
Histidine	31. 0	25. 8
Lysine	14. 6	97. 3
Arginine	51. 0	51. 0

of serine as much as 735μg and the lowest amount of methionin as much as 4.9μg. On the other hand, 316.7mg dry weight of *Porphyra* cultured at 20°C for 8 days released amino acids containing the highest amount of serine as much as 185.5μg and the lowest amount of isoleucine as much as 17.5ug. The amounts of amino acids fluctuated depending on temperature, but their relative compositions were always unchanged. Asparatic acid, glutamic acid, glycine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine were higher in amount when *Porphyra* was cultured at 20°C for 8 days than at 10°C for 23 days (Table 31, Figs. 3, 4 and 5).

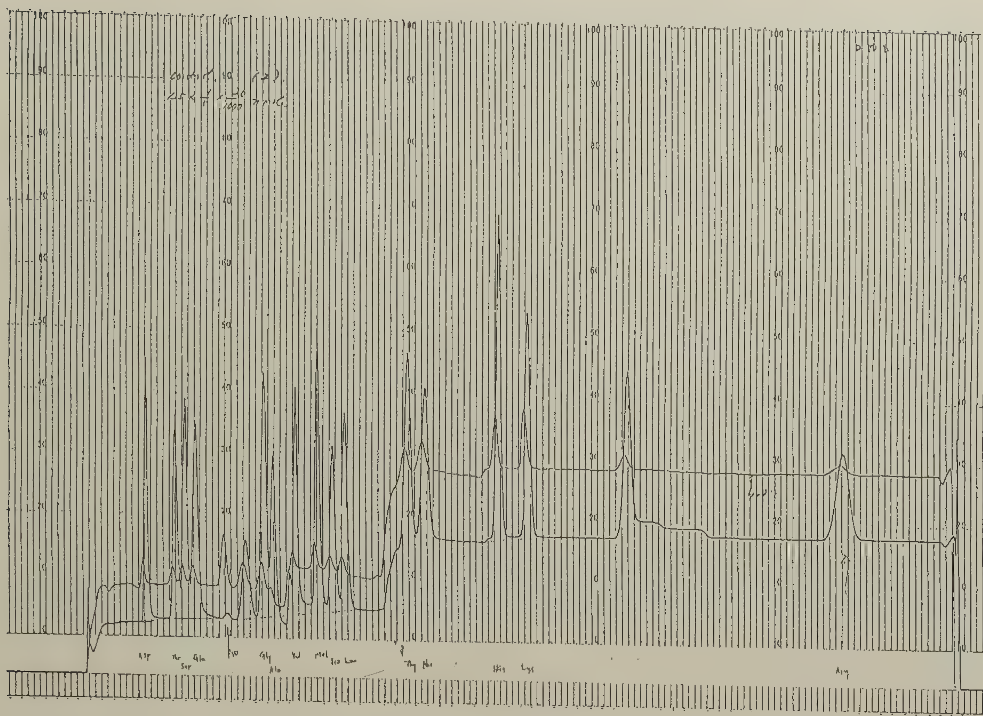


Fig. 3. Chromatogram of amino acids mixture (5 n mole).



Fig. 4. Chromatogram of amino acids in the medium in which *Porphyra* species grow at 10°C.

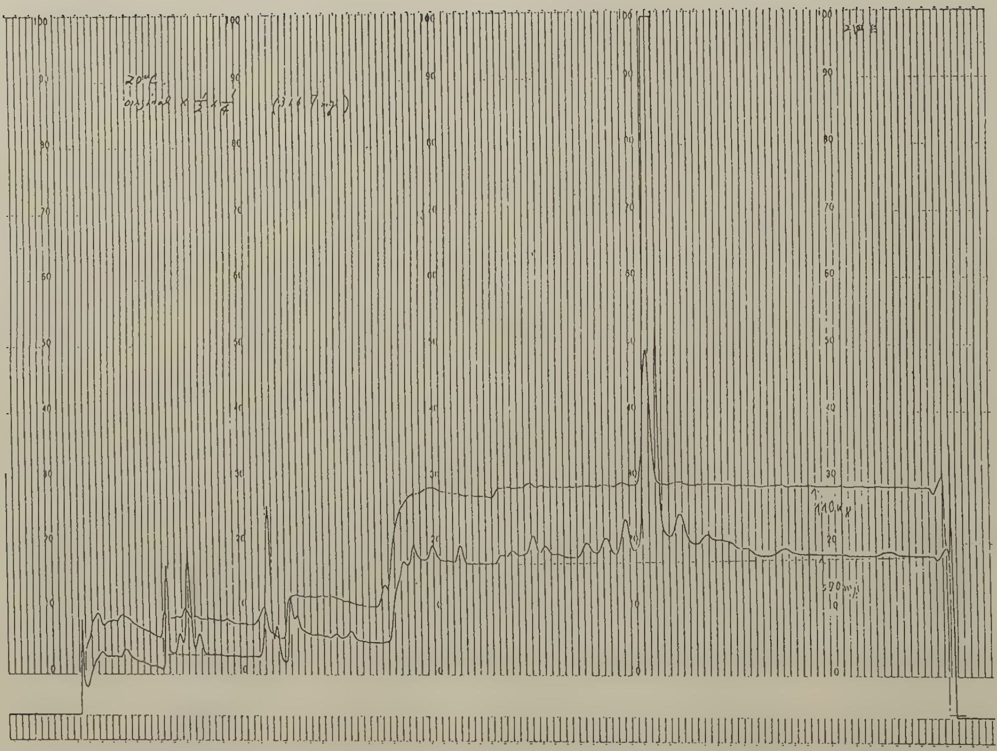


FIg. 5. Chromatogram of amino acids in the medium in which *Porphyra* species grow at 20°C.

Discussion

FOGG and WESTLAKE (1955) reported that several unicellular fresh water algae discharged some amino acids into ambient water. FOGG (1962) also found that some amino acids existed in extracellular metabolites liberated by algae. However, amino acids were not fractionated in above two references. The present study is the first to show that *Porphyra* liberates amino acids and that they are fractioned in detail. Amino acids secreted by *Porphyra* include almost all amino acids which have been known so far. The quantities of the amino acids discharged changed depending on enviromental conditions and increased at higher temperature like 20°C which was too high and unfavorable for *Porphyra* to grow. It is interesting to note that asparatic and glutamic acids, amino acids favoring the growth of *Beneckea* sp., one of the pathogens causing white rot, are contained in the extracellular amino acids secreted at higher temperature by *Porphyra*.

Chapter 10. Effect of nutrients on the growth of *Porphra* in bacteria-free culture

It is known that a deficiency of organic substances slows growth of *Porphyra*, apparently as a result of physiological upsets (Author's unpublished data). On the other hand, bacteria attached to *Porphyra* utilize amino acids for their growth and discharge carbon dioxide, as described in Chapters 6 and 7. Therefore, in the present study *Porphyra* was grown in axenic cultures so as certain the requirement of organic nutrients and the effects of carbonic acid substances for its growth. Carbonic acid substances includes the following four chemicals in the present study: dissolved carbon dioxide, carbonate ion, bicarbonate ion and carbonic acid.

Bacteria-free cultures of unicellular algae have been grown by LEWIN (1959), MCDANIEL *et al.* (1961), BROWN and BISHOFF (1962) SOLI (1964), PRINGSHEIM (1964), TOKUDA (1966) and STEIN (1973). Multicellular algae have been grown in axenic culture by Provasoli on *Ulva* (1958) by TATEWAKI and PROVASOLI on three species of *Antithamnion* (1963) and by FRIES and PETTERSSON on several red algae (1968).

Materials and methods

1) Algal materials

The following three species were employed in the present study.

a) *Porphyra leucosticta*, harvested from a stock culture maintained in a round-bottom 500ml flask with aeration using artificial seawater (Table 1).

b) *Porphyra yezoensis*, collected from population growing on inshore rocks near the Nansei Regional Fisheries Research Laboratory.

c) *Porphyra tentra*, collected at Matsukawa-ura, Miyagi-pref.

2) Purification

The attainment of bacteria-free culture of *Porphyra* was difficult, because of microorganisms in its mucilagenous surface layer. Surface sterilization was utilized using exclusively a mixture of the anti-biotics. The thalli were cut into 25mm² pieces. The pieces were dipped and dragged for 30-35cm through artificial seawater (Table 1) solidified with 1% agar. Then the pieces were buried for two weeks in a petri plate containing 50ml artificial seawater mixed with penicillin G in a dose of 40,000 units and streptomycin sulfate in a dose of 20mg (pot.) solidified with 1% agar.

Another method of eliminating microorganisms was also used. Pieces of thallus was placed for overnight in 50ml seawater containing 20,000 unit of penicillin G, 10mg (Pot.) of strepto-

Table 32. Component of the enrichment used.

Pure water	100 mℓ
$NaNO_3$	350 mg
Na_2glycero PO_4	50 mg
FeCl $6H_2O$	12.1mg
Metal solution	25 mℓ
Vitamin B_{12}	10 γ
TRIS	500 mg
Metal solution 1 mℓ=	
H_3BO_3	0.2 mg
$FeCl_3$ $6H_2O$	0.048mg
$MnCl_2$ $4H_2O$	0.144mg
$CoCl_2$ $6H_2O$	0.04 mg
$ZnCl_2$	0.01 mg
Na_2 EDTA	1 mg

mycin sulfate and 2ml of ES medium (Table 32), after the "dip and drag" treatment. These procedures were repeated several times to obtain perfect sterilization.

Axenic culture of juvenile *Porphyra* plants 1mm long were obtained by soaking for two days in a test tube containing 30ml of artificial seawater mixed with 200,000 units of penicillin G and lg (Pot.) of streptomycin sulfate, and was shaked violently in the test tube to eliminate attached microorganisms. After this procedure was repeated three times, the thallus was removed from the test tube to a Petri dishes containing bacterial media and was cultured for 5-7 days. Then the thallus free from bacteria was transferred into a sterilized media. The last mentioned technique was most successful to obtain clean thalli.

3) Sterility test

The sterility test media used were ST_3 (TATEWAKI and PROVASOLI 1963) and E_6 (PROVASOLI *et al.* 1956) and the establishment of axenic culture was examined one month later.

4) Culture of *Porphyra*

In experiments 1, 2, 3 and 4, a piece of sterilized 25mm² *Porphyra* was cultured in a 20ml L test tube containing 10ml of medium using a culture apparatus of Monad. The cultures were maintained at 16 ±0.5°C under a 9: 15 LD cycle. The light intensity was 5,000 lux at the surface of the test tube. In experiments 5 and 6, a sterilized thalli 1mm long were each cultured in 125ml, Erlenmeyer flasks containing 50ml of media using a shaking culture apparatus. The cultures were maintained at 17± 2°C under a 9: 15 LD cycle. The light intensity was 3,000 lux at the surface of the flask. A modified artificial seawater described in Materials and Methods of Chapter 8 was used as a control.

Besides the above experiments, the nutritive requirement for the growth of *Porphyra* was examined using the following substances; Vitamines: thiamine, riboflavin, pyridoxine, pyridoxamine, cyanocobalamine, orotic acid, ascorbic acid, pantothenate, nicotinic acid, inositol, thymine, folic acid, putrescine, p-aminobenzoic acid, folinic acid, choline, biotin, amino acids: aspartic acid, glutamic acid, alanine, valine, arginine, lysine, leucine, isoleucine, serine, thyrosine, phenylalanine, threonine, tryptophane, histidine, proline, glycine, cystine, taurine, purine pyrimidine: guanine, cytosine, adenine, uracil, phytohormones: indol acetic acid, gibberellin.

At the end of experiment, plants were observed for colour, shape and the state of the growth and their length was measured.

Results

The effect of organic substances on the growth of *Porphyra* were not detected, though its growth seemed to be promoted by some amino acids. Therefore, it is uncertain whether organic substances used in the present experiment were required for the growth of *Porphyra*.

Results obtained are shown in Tables 33-38. In experiment 1 (Table 33), the media

Table 33. Growth of *Porphyra* in axenic culture using artificial sea water containing Tris (Experiment 1).

Addition mg/100mℓ	Remarks on growth	
	14 days	30 days
None	Reddish but growing	Reddish but growing
Tris 50	Normal and growing	Normal and growing

were replaced once 2 weeks later. The growth of *Porphyra* was better in the medium containing tris (hydroxymethyl) aminomethane (Tris) than the control. In experiment 2 (Table 34), media were not replaced. At the end of 19 days, *Porphyra* grew better and had

Table 34. Growth of *Porphyra* in axenic culture using artificial sea water containing Tris (Experiment 2).

Addition mg/100mℓ	Remarks on growth		
	9 days	16 days	19 days
None	Reddish but growing	Reddish but growing	Pale 5 mm long
Tris 50	Normal and growing	Normal and growing faster	Normal 1cm long

more natural colour in the medium containing Tris than the control. In experiment 3 (Table 35), the media were replaced once 3 weeks later. At the end of 30 days, the growth of

Table 35. Growth of *Porphyra* in axenic culture using artificial sea water containing Tris and sodium bicarbonate (Experiment 3).

Addition mg/100mℓ	Remarks on growth		
	3 days	21 days	30 days
None	Growing	Growing	No growing but alive
Tris 50 NaHCO 16.8	Growing	Growing faster	Normal and growing 3 cm long

Table 36. Growth of *Porphyra* in axenic culture using artificial sea water containing Tris and sodium bicarbonate (Experiment 4).

Addition mg/100mℓ	Remarks on growth		
	3 days	9 days	21 days
None	Growing	Normal and growing	Growing
Tris 50 NaHCO 16.8	Growing	Normal and growing	Growing faster

Table 37. Growth of *Porphyra* in axenic culture using artificial sea water (Experiment5).

Addition mg/100mℓ	Remarks on growth 39 days	44 days
None	Growing	Growing 5 mm long

Table 38. Growth of *Porphyra* in axenic culture using artificial sea water (Experiment 6).

Addition mg/100mℓ	Remarks on growth 10 days	14 days	22 days
None	Growing	Growing	Growing 5 mm long

Porphyra was better in the medium containing Tris and carbonic acid substances which were twice the concentration of artificial seawater. In experiment 5 and 6 (Table 37 and 38), the medium was exclusively modified artificial seawater used as control in other experiments and was not replaced. At the end of 44 days, *Porphyra* did not grow well and attained a length of only 5mm in both cases.

Discussion

Though antibiotic purification of *Porphyra* was not easy to establish, it became evident that better results were obtained when juvenile thalli less than 1mm long were used. In most cases, *Flavobacterium/Cytophaga* resisted to be eliminated in the pure culture. On the other hand, from the author's unpublished data, it was found that *Flavobacterium/Cytophaga* became undetected in bacteria flora on *Porphyra* within two weeks and *Pseudomonas* or *Vibrio* predomonated instead when casamino acid was added to the medium. Therefore it might be better to sterilize aseptically *Porphyra* after changing composition of bacterial flora.

Porphyra attained merely to the length of 3 cm at its maximum, as the culture vessel was not big enough for its growth. In addition, *Porphyra* retarded to grow and became pale when enough carbonic acid substances were not provided. However it could be said that *Porphyra* grows healthy in the bacteria-free culture provided the culture vessel is shaked and the medium is replaced during the experiment. Shaking is assumed to play an important role in supplying carbonic acid substances to *Porphyra*. The carbonic acid substances are also thought to be one of factors indispensable for the growth of *Porphyra* in the bacteria-free culture.

From the above results, it might be concluded that *Porphyra* grows well in the presence of carbon dioxide released by the attached bacteria.

Chapter 11. General discussion

SUTO *et al.* (1972) reviewed generally the diseases in *Porphyra* farming, describing the infectious white wasting disease as white rot. In order to detect the pathogens of white rot, the attached bacteria were isolated from healthy *Porphyra* and their biological and biochemical characteristics were investigated. Each isolate was identified to generic level according to the scheme described by Shewan *et al.* (1960a). The microflora on healthy *Porphyra* plants which had been cultured *in vitro* for several years consisted of *Micrococcus*, *Achromobacter*, *Flavobacterium/Cytophaga* and *Pseudomonas* and these bacteria, except *Micrococcus*, were often detected. On the other hand, *Micrococcus*, *Achromobacter*, *Flavobacterium/Cytophaga*, *Pseudomonas*, *Aeromonas* and yeast were isolated from healthy *Porphyra* growing on rocks or "Hibi". The common genera which were detected on naturally growing *Porphyra* were also the same as described on *Porphyra* growing *in vitro*. Accordingly, it can be concluded that *Achromobacter*, *Flavobacterium/Cytophaga* and *Pseudomonas* were the dominant genera of bacteria associated with healthy *Porphyra*.

Beneckea sp. was isolated from diseased *Porphyra* having the symptoms of white rot. The cells of diseased *Porphyra* became white and died, and the plants finally sloughed off from "Hibi". After examining the relationships between environmental factors and the occurrence of white rot, it was found that *Beneckea* sp. thrived on *Porphyra* when the thalli were exposed for more than 4 hours at dawn and a discrepancy of 8°C or more existed between the atmospheric and seawater temperatures. It is not clear whether this big discrepancy corresponds with the rise of seawater temperature or not. These factors seemingly responsible for outbreaks of white rot were considered to be a part of many primary causes. When *Beneckea* sp. were inoculated to healthy *Porphyra*, the cells became homogeneous and finally deteriorated, showing the symptom of white rot. *Beneckea* sp. was always isolated again from infected *Porphyra* in which white rot was caused experimentally. Therfore, *Beneckea* sp. was considered to be one of pathogens causing white rot.

The bacteria associated with *Porphyra* were tested for their nutrient requirement. Generally speaking, all bacteria attached to *Porphyra* utilized amino acids for their growth. Especially *Achromobacter*, *Flavobacterium/Cytophaga* and *Pseudomonas*, common members of bacterial flora on healthy *Porphyra*, used many amino acids, but *Beneckea* sp. grew well exclusively in the presence of alanine, aspartic acid and glutamic acid. As for the effects of temperature, *Beneckea* sp. grew well over 20°C, whereas *Flavobacterium/Cytophaga* grew slowly at this temperature. From the sodium chloride requirement, it was conceivable that *Micrococcus* and *Flavobacterium/Cytophaga* were of terrestrial origin, *Achromobacter* and *Pseudomonas* were marine bacteria and *Beneckea* sp. was halophilic. Considering the results obtained above,

Beneckea sp. seemed different from other attached bacteria studied in its nutrient requirements and the way it adapted to environmental factors.

It has been known that planktonic unicellular algae discharge organic substances but the fact that *Porphyra* also does so was here reported for the first time. The organic carbon produced by *Porphyra* during photosynthesis amounted as much as 0.6-4.1% of the inorganic carbon supplied.

Porphyra also released many kinds of amino acids to a medium composed exclusively of inorganic nutrients and the liberation of the mine amino acids, aspartic acid, glutamic acid, glycine, leucine, tyrosine, pheylalanine, histidine, lysine and arginine, was stimulated as temperature rose. Amino acids excreted by *Porphyra* were utilized by bacteria, leading to the production of carbon dioxide. However, the types of utilized amino acids and the amounts of released carbon dioxide differed among the bacteria. *Beneckea* sp. utilized well proline, glutamic acid, aspartic aicd, alanine and serine, whereas *Flavobacterium/Cytophaga* used only serine, leucine and threonine. *Porphyra* did not grow by providing any amino aicds, nucleic acids, and phytohormones when grown in axenic culture. On the contrary, carbon dioxide in amounts twice as high as in the artificial seawater used in the present study supported normal growth of *Porphyra*. It is clear from the above result that axenic *Porphyra* needs more carbon dioxide than when cultured in a medium contaminated with bacteria.

Porphyra grew well in the presence of carbon dioxide released by the attached bacteria and the extracellular amino acids of *Porphyra* also stimulated the growth of the bacteria. In other words, *Porphyra* provides amino acids to the attached bacteria and the bacteria produce carbon dioxide which stimulates the growth of *Porphyra*.

JOLLY and JONES (1977) reported a symbiotic relationship between *Navicula* and *Flavobacterium*. They speculated that the metabolite produced by the bacterium might work as a chelator to nourish the growth of the diatom. ALLEN (1977) considered that there was a mutual exchange of nutrients between aquatic plants and the attached bacteria with the result increased productivity in the lake. He also speculated that the amount of nutrients taken in by the diatoms attached to the aquatic plants increased with a greater availability of carbon dioxide produced by bacteria. The literature cited above suggests that a symbiotic relationship occurs exclusively between unicellular algae and the attached bacteria; there is so far no direct proof. However, now the first time a symbiotic relationship is indicated between *Porphyra* and the attached bacteria in relation to the liberation of amino acids and the production of carbon dioxide.

The outbreak of white blight disease is said to be triggered by unfavorable environmental factors as follows: (1) the seawater temperature becomes higher than usual, especially higher at the days between 50 and 70 from the beginning of cultivation in the sea (KATADA 1967);

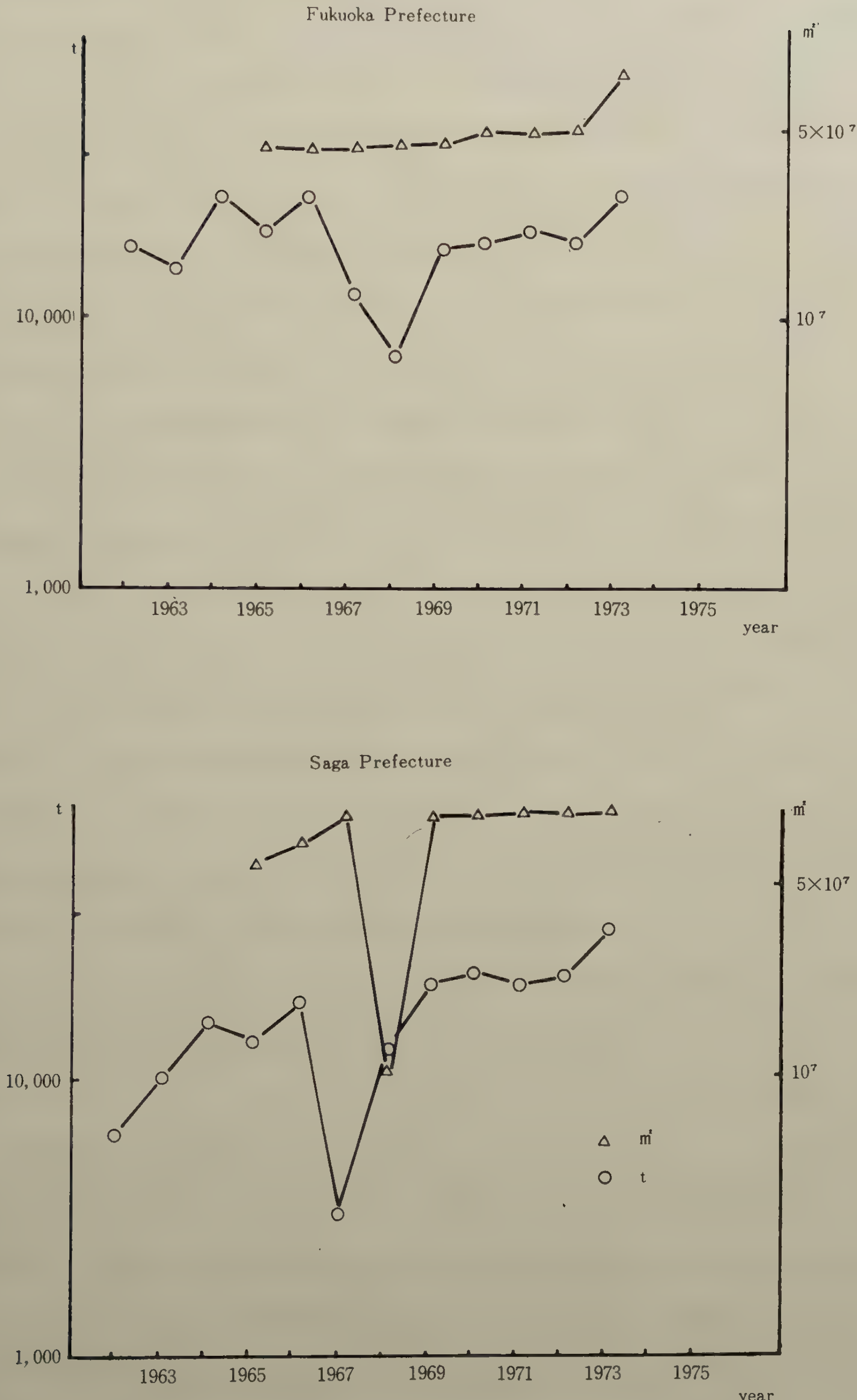


Fig. 6. Nori farming area and annual production.

(2) a shortage of nutrients like carbonic acid substances for the normal metabolism of *Porphyra* (KATADA 1967) and (3) when the pH increases due to stagnating seawater, resulting in the low metabolism of *Porphyra* (WATANABE *et al.* 1968).

As mentioned above, *Porphyra* utilizes carbon dioxide produced by bacteria; it needed more carbonic acid substance when grown in axenic culture. It was also shown that *Beneckea* sp decomposed aspartic and glutamic acids which were liberated by *Porphyra* at high temperature. Therefore, *Beneckea* sp. probably thrives when these 2 amino acids exist on the surfaces of *Porphyra* at higher seawater temperatures. In summary, *Porphyra* becomes unhealthy when oceanographic and meteorological conditions change and the plants exude abnormally amino acids which are readily available for *Beneckea* sp. ; the result is white rot.

The white wasting-like disease contains 2 disorders, the white blight and the white rot. When the 2 diseases occur together, much damage occurs in the *Porphyra* industry. Remarkable drop in the annual crops were shown in Saga Prefecture in 1967 and in Fukuoka Prefecture in 1967 and 1968. It is assumed that the drops were caused by bacteria and physiological disorders (Fig. 6).

Another disease caused by bacteria was described by ANDO and INOUE (1961) on *Laminaria japonica* var. *ochotensis*, in Wakkanai, northern Hokkaido. Experiments included the inoculation of *Vibrio* sp. which had been isolated from diseased *Laminaria*. A deteriorated spot, 1 mm in diameter, produced after 1 day, developed into a hole after incubation of inoculated *Laminaria* for another 2 days. They concluded that the disease was caused by alginase produced as the bacteria multiplied on *Laminaria*.

Seaweed diseases have been known to be caused by biological and physiological factors, but there has been little information about their pathology (ANDREWS 1967). There remain many unsolved problems of host-parasite interactions, except in some diseases of *Porphyra*, such as the relationships between *Porphyra* and *Pythium Porphyrae* in the red rot.

The present study shows for the first time that white rot disease of *Porphyra*, is probably caused by *Beneckea* sp.

Literature cited

AARONSON, S., 1971 : The synthesis extracellular macromorecules and membranes by a population of the phytoflagellate *Ochromonas danica*. Limnol. Oceanogr. **16**, 1-9.

AKASAKA, Y., 1956 : On the bud blight of the *Porphyra* in Kesenuma bay and Mangoku-Ura bay of Miyagi Prefecture. The Aquaculture, **4**, 41-55. (In Japanese).

AKASAKA, Y., T. HATAKEYAMA and T. HENMI, 1961 : Research on the prevention of the *Porphyra* disease in Kesenuma bay. Research report on the prevention of the *Porphyra*

disease in Tohoku district in Japan. The meeting on the prevention of the *Porphyra* disease in Tohoku district in Japan, 37-55. (In Japanese).

ALLEN, H. L., 1971 : Primary productivity, chemo-organotropy, and nutritional interactions of epiphytic algae and bacteria on macrophytes in the littoral of a lake. Ecol. Monogr. **41**, 97-127.

ALLEN, R. D. and P. BAUMANN, 1971 : Structure and arrangement of *flagella* in species of the genus *Beneckea* and *Photobacterium* fischeri. J. Bacteriol. **107**, 295-302.

ANDO, Y. and K. INOUE, 1961 : Bacteria, capable of decomposing brown algae *Laminaria*., Bull. Japan. Soc. Phycol., **9**(1), 17-21. (In Japanese).

ANDREWS, J. H., 1976 : The pathology of marine algae. Biol. Rev., **51**, 211-253.

ARASAKI, S., 1947 : Studies on the wasting disease of the cultured lavers (*Porphyra tenera*). Bull. Japan. Soc. Sci. Fish., **13**(3), 74-90. (In Japanese).

ARASAKI, S., 1956 : A disease and its prevention of *Porphyra tenera*. Shokubutsu Boeki, 10, 243-246. (In Japanese).

ARASAKI, S., 1960 : A chytridean parasite on the *Porphyra*. Bull. Japan. Soc. Sci. Fish., **26**, 543-548. (In Japanese).

ARASAKI, S., A. Inoue and Y. Kochi, 1960 : The disease of the cultured *Porphyra*, with special reference to the cancer-disease and the chytrid-disease which occurred at the culture field in Tokyo bay during 1959-1960. Bull. Japan. Soc. Sci. Fish., **26**(11), 1074-1079. (In Japanese).

ARASAKI, S., 1962 : Study on the artificial culture of *Porphyra tenera* Kjellm. III. On the red wasting disease of *Porphyra*, especially on the physiology of the causal fungus *Phythium* sp. nov. Bull. Agricull. Fectri. Lab., **3**, 87-93. (In Japanese).

ARASAKI, S., K. AKINO and T. TOMIYAMA, 1968 : A comparison of some physiological aspects in a marine *Phythium* on the host and on the artificial medium. Bull. Misaki Mar. Biol. Inst. Kyoto Univ., **12**, 203-206.

BAUMANN, P., L. BAUMANN and M. MANDEL, 1971a : Taxonomy of marine bacteria. The genus *Beneckea*. J. Bacteriol., **107**, 268-294.

BAUMANN, P., L. BAUMANN, M. MANDEL and R. D. AILEN, 1971b : Taxonomy of marine bacteria. *Beneckea nigrpulchrituda* sp. n. J. Bacteriol., **108**, 1380-1383.

BAUMANN, P., L. BAUMANN and J. L. REICHEIT, 1973 : Taxonomy of marine bacteria. *Beneckea parahaemolytica* and *Beneckea alginolytica*. J. Bacteriol., **113**, 1144-1155.

BERLAND, B. R., M. G. BIANCHI and S. Y. MAESTRINI, 1969 : Etude des bacteries associees aux Algues marines en culture. I. Determination preliminaire des especes. Mar. Biol., **2**, 350-355.

BERLAND, B. R., D. J. BONIN and S. Y. MAESTRINI, 1970 : Study of bacteria associated with

marine algae in culture. III. Organic substrates supporting growth. Mar. Biol., **5**, 68-76.

BERMAN, T. and O. Holm-HANSEN, 1974: Release of photoassimilated carbon as dissolved organic matter by marine phytoplankton. Mar. Biol., **28**, 305-310.

BROCK, T. D., 1966: Principles of microbial ecology. Prentice-Hall, Inc./Englewood Cliffs, New Jersey.

BROWN, R. M. and H. W. BISCHOFF, 1962: A new and useful method for obstaining axenic cultures of algae. Phycol. News Bull., **15**, 43-44.

CHAN, R. C. S. and E. A. MCMANUS 1969: Distribution, characterization and nutrition of marine micrioorganisms from the algae *Polysiphonia lanosa* and *Ascophyllum nodosum*. Can. J. Microbiol., **15**, 409-420.

CHIBA PREFECTURE NAIWAN FISHERIES EXPERIMENT STATION, 1968: Research and study report on the prevention of industrial damage in the farming *Porphyra* subsidized by the central government. 1967 fiscal year, 4-8. (In Japanese).

FISHERY EXPERIMENT STATION OF THE GOVERNMENT-GNERAL OF CHOSEN, 1934: On the poor production in *Porphyra* farming at Rakuyoko. Bull. Fish. Experi. Sta. Government-Gen. Chosen, **7**, 1-29. (In Japanese).

FOGG, G. E. and D. F. WESTLAKE, 1955: The importance of extracellular products of algae in freshwater. Verhandlungen der Internationalen Vereinigung fur Theoretische und Angewandte Limnologie. **12**, 219-232.

FOGG, G. E., 1962: Extracellular products. In "Physiology and biochemistry of algae". ed. R. A. Lewin, Academic Press Inc. New York and London, 475-489.

FRIES, L., 1963: On the cultivation of axenic red algae. Physiologia plantarum, **16**, 695-708.

FRIES, L., 1966: Influence of iodine and bromine on growth of some red algae in axenic culture. Physiologia plantarum., **19**, 800-808.

FRIES, L. and H. PETTERSSON, 1968: On the physiology of the red alga Asterocytis ramosa in axenic culture. Br. phycol. Bull., **3**, 417-422.

FRIES, L. 1970: The influence of microamounts of organic substances other than vitamins on the growth of some red algae in axenic culture. Br. phycol. J., 5, 39-46.

FUKUOKA PREFECTURE ARIAKE FISHERIES EXPERIMENT STATION, 1968: Research and study report on the prevention of industrial damage in the farming *Porphyra* subsidized by the central government, 1967 fiscal year, 107-146. (In Japanese).

FUKUOKA PREFECTURE ARIAKE FISHERIES EXPERIMENT STATION, 1969: Research and study report on the prevention of industrial damage in the farming *Porphyra* subsidized by the central Government, 1968 fiscal year, 134-161. (In Japanese).

FUKUOKA PREFECTURE ARIAKE FISHERIES EXPERIMENT STATION, SAGA PREFECTURE AQUACULTURE EXRERIMENT STATION, KUMAMOTO PREFECTURE LAVER RESEARCH LABORATORY AND

NAGASAKI PREFECTURE FISHERIES EXPERIMENT STATION, SHIMABARA BRANCH, 1970: Research and study report on environmental survey in Ariake sea, 1969 fiscal year. (In Japanese).

FUJIKAWA, K, 1937: On the physiology of the *Porphyra* in Chosen, Sotokufu, Bull. Fish. Experi. Sta. Government-Gen. Chosen, **8**, 1-131. (In Japanese).

FUJITA, Y., B. ZENITANI, Y. NAKAO and T. MATSUBARA, 1972: Bacteriological studies on diseases of cultured laver-II. Bacteria associated with diseased laver. Bull. Japan. Soc. Sci. Fish., **38**(6), 565-569. (In Japanese).

FUJITA, Y., 1973: Maceration of laver frond by enzymes of bacteria causing green spot rotting-like deterioration. Bull. Japan. Soc. Sci. Fish., **39**(8), 911-915. (In Japanese).

FUJITA, Y. and B. ZENITANI, 1976: Studies on pathogenic *Pythium* of laver red rot in Ariake Sea farm-I. General mycological characteristics. Bull. Japan. Soc. Sci. Fish., **42**(10), 1183-1188. (In Japanese).

FUJITA, Y. and B. ZENITANI, 1977a: Studies on pathogenic *Pythium* of laver red rot in Ariake Sea farm-II. Experimental conditions and nutritional requirements for growth. Bull. Japan. Soc. Sci. Fish., **43**(1), 89-95. (In Japanese).

FUJITA, Y. and B. ZENITANI, 1977b: Studies on pathogenic *Pythium* of laver red rot in Ariake Sea farm-III. Environmental and nutritional conditions for production of sexual organs. Bull. Japan. Soc. Sci. Fish., **43**(8), 921-927. (In Japanese).

FUJITA, Y. and B. ZENITANI, 1977c: Studies on pathogenic *Pythium* of laver red rot in Ariake Sea farm-IV. Serological differentiation of pathogenic *Pythium* strains. Bull. Japan. Soc. Sci. Fish., **43**(11), 1313-1318. (In Japanese).

FUJITA, Y., 1978: Studies on pathogenic *Pythium* of laver red rot in Ariake Sea farm-V. Germination of *Pythium porphyrae* Oospores. Bull. Japan. Soc. Sci. Fish., **44**(1), 15-19. (In Japanese).

FUJIYAMA, T., 1957a: A chytrid disease of *Porphyra*. The Aquaculture, **4**, 69-73. (In Japanese).

FUJIYAMA, T., 1957b: Cyto-chemical research on a chytrid disease of *Porphyra tenera*. Suisan-gaku Shusei, Todai Shuppankai, 829-840. (In Japanese).

GIRARD, A. E., J. D. BUCK and B. J. COSENZA, 1968: A nutritional study of some agarolytic marine bacteria. Can. J. Microbiol., **14**, 1193-1198.

HELLEBUST, J. A., 1974: Extracelluar products. In "Algal physiology and biochemistry". ed. W. D. P. STEWART, Blackwell Scientific Publication, 838-863.

HIDAKA, T. and M. SAKAI, 1968: Comparative observation of the inorganic salt requirements of the marine and terrestrial bacteria. Proceedings of the U.S.- Japan Seminar on Marine Microbiology, 125-149.

HILL, L. R., 1968: The determination of deoxyribonucleic acid base compositions and its

application to bacterial taxonomy. In "Identification method for microbiologists part B". ed. B. M. GIBBS and D. A. SHAPTON, Academic press. London and New York. 177-186.

ISHIO, S., T. YANO and R. NAKAGAWA, 1971 : Algal cancer and causal substances in waters from the coal chemical industry. Proceeding of the 5th International Water Pollution Research Conference. III. 18, 1-8.

ISHIO, S., K. KAWABE and T. TOMIYAMA, 1972 : Algal cancer and its causes-I. Carcinogenic potencies of water and suspended solids discharged to the river Ohmuta. Bull. Japan. Soc. Sci. Fish., **38**(1), 17-24. (In Japanese).

ISHIO, S., H. NAKAGAWA and T. TOMIYAMA, 1972 : Algal cancer and its causes-II. Separation of carcinogenic compounds from sea bottom mud polluted by wastes of the coal chemical industry. Bull. Japan. Soc. Sci. Fish., **38**(6), 571-576. (In Japanese).

ITURRIAGA, R. and H. G. HOPPE, 1977 : Observations of heterotrophic activity on photoassimilated organic matter. Mar. Biol., **40**, 101-108.

JOLLEY, E. T. and A. K. JONES, 1977 : The interaction between *Navicula muralis* Grunow and an associated species of *Flavobacterium*. J. Phycol., **12**, 315-328.

KATADA, M., 1967 : On the stability and increase in production of *Porphyra*. Watakushitachi-no-Norikenkyu, 64-73. (In Japanese).

KATO, T., 1967 : Studies on the yellow spot disease of conchocelis in *Porphyra*. Bull. Tokushima Pref. Fish. Experi. Sta., **10**, 1-66. (In Japanese).

KATO, S., T. WATANABE and Y. AKASAKA, 1970 : Studies on the diseases of cultural *Porphyra*-II. Physiological changes of *Porphyra* frond until white wasting disease like occur. Bull. Japan. Soc. Sci. Fish., **36**(10), 1001-1006. (In Japanese).

KATO, S. and T. WATANABE, 1971 : Studies on the diseases of cultured *Porphyra*-III. Phosphorus metabolism in *Porphyra*, manifesting the white wasting like symptom. Bull. Japan. Soc. Sci. Fish., **37**(5), 380-386. (In Japanese).

KATO, S. and T. WATANABE, 1972 : Studies on the diseases of cultured *Porphyra*-V. On the DNA of cancered *Porphyra* cells. **38**(7), 693-697. (In Japanese).

KATO, S., T. WATANABE and Y. SATO, 1973a : Studies on the diseases of cultured *Porphyra*-VI. Nutritional behavior of the causal fungus of the red wasting disease of Nori. Bull. Japan. Soc. Sci. Fish., **39**(7), 771-775. (In Japanese).

KATO, S., WATANABE and Y. SATO, 1973b : Studies on the diseases of cultural *Porphyra*-VII. A comparison of physiological properties among the different isolates of the causal fungus of the red wasting disease. Bull. Japan. Soc. Sci. Fish., **39**(8), 859-865. (In Japanese).

KAWABE, K., 1962 : Studies on the tumour disease in *Porphyra* tenera-I. Bull. Fukuoka Pref. Ariake Fish. Experi. Sta., 1961 fiscal year, 33-60. (In Japanese).

KAWABE, K. and A. MIZUNO, 1963 : Studies on the tumour disease in *Porphyra tenera*-II. Bull.

Fukuoka Pref. Ariake Fish. Experi. Sta., 1962 fiscal year, 153-173. (In Japanese).

KAWABE, K., A. MIZUNO and M. Inoue, 1967: On the initiation, development and prevention study on tumour disease of *Porphyra* and physiological abnormality of *Ruditapes philippinarum* in Ohmuta. Bull. Fukuoka Pref. Ariake Fish Experi. Sta., 1966 fiscal year, 1-24. (In Japanese).

KHAILOV, K. M., 1973: Ecological metabolism in the ocean (Translated from Russian by N. TSURU), Tsukiji-Shokan, Tokyo, viii+224pp.

KITO, H., 1972: Electron microscopic observations on the tumour in *Porphyra*. Bull. Tohoku Reg. Fish. Res. Lab., 32, 77-82. (In Japanese).

KOMAGATA, K., 1975: Measurement of basic composition in DNA. In "Classification and identification of microorganisms". ed. T. HASEGAWA, Tokyo Shuppankai, Tokyo, 426-432. (In Japanese).

KONG, M. K. and K. CHAN, 1979: A study on the bacterial flora isolated from marine algae. Bot. Mar., **12,** 83-97.

KOVACS, N., 1956: Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature, **178,** 703.

KUMAMOTO PREFECTURE LAVER RESEARCH LABORATRY, 1969: Research and study report on environmental survey in Ariake Sea, 1968 fiscal year. (In Japanese).

LAYCOCK, R. A., 1974: The detrital food chain based on seaweeds-I. Bacteria associated with the surface of *Laminaria* fronds. Mar. Biol., **25,** 223-231.

LEFEVRE, M., 1964: Extracellular products of algae. In "Algae and man". ed. D. F. Jackson, Plnum Press, New York, 337-367.

LEIFSON, E., 1963: Determination of carbohydrate metabolism of marine bacteria. J. Bacteriol., **85,** 1183-1184.

LEWIN, R. A., 1959: The isolation of algae. Rev. Algol., **3,** 181-198.

MACDANIEL, H. R., J. B. M. BROCK and R. O. BOWMAN, 1961: Isolation of pure cultures of algae from contaminated cultures. Appl. Microbiol., **10,** 223.

MIGITA, S., 1969: Olpidiopsis disease of culture *Porphyra*. Bull. Fac. Fish. Nagasaki Univ., 28, 131-145. (In Japanese).

MIYAGI PREFECTURE FISHERIES EXPERIMENT STATION, 1968: Research and study report on extensive aquaculture technique of suitable organisms in coastal area (*Porphyra* sp.) subsidized by the central government, 1965-1966 fiscal year, 8. (In Japanese).

MIYAGI PREFECTURE FISHERIES EXPERIMENT STATION, KESENUMA BRANCH, 1979: Research and study report on the prevention of industrial damage in the farming *Porphyra* subsidized by the central government. 1968 fiscal year, 1-26. (In Japanese).

MIYAGI PREFECTURE FISHERIES EXPERIMENT STATION, KESENUMA BRANCH, 1970: Research and

study report on the prevention of industrial damage in the farming *Porphyra* subsidized by the central government. 1969 fiscal year 1-42. (In Japanese).

Moebus, K. and K. M. Johnson, 1974: Exudation of dissolved organic carbon by brown algae, Mar. Biol., **26**, 117 125.

Murchelano, R. A. and C. Brown, 1969: Bacterial flora of some algal foods used for rearing bivalve larvae. J. Fish. Red. Board Can., **26**, 2760-2764.

Nakao, Y., T. Onohara, T. Matsubara, Y. Fujita and B. Zenitani, 1972: Bacteriological studies on diseases of cultured laver-I. Green spot rotting-like deterioration of laver frond by bacteria, *in vitro*. Bull Japan. Soc. Sci. Fish., **38**(6), 561-564. (In Japanese).

Nozawa, K. and Y. Nozawa, 1955: Studies on the protoplasm of algae-I. Bull. Japan. Soc. Sci. Fish., **20**(10), 873-880. (In Japanese).

Nozawa, K. and Y. Nozawa, 1957: Studies on the protoplasm of algae-II. On the "Perforating disease" of laver. Bull. Japan. Soc. Sci. Fish., **22**(11), 694-700. (In Japanese).

Nozawa, Y. and K. Nozawa, 1957: Studies on a disease of conchocelis-phase of *Porphyra* (Preliminary notes). Bull. Japan. Soc. Sci. Fish., **23**(7 and 8), 427-429. (In Japanese).

Ohmi, H., 1963: On epiphytic species of *Porphyra* and their hosts. Bull. Japan. Soc. Phycol., **11**(1), 38-44. (In Japanese).

Okamura, K., 1909: Asakusanori, Hakubunkan, 105-111. (In Japanese).

Pringsheim, E. G., 1964: Pure cultures of algae. Their preparation and maintenance. Hafner Publishing Company. New York and London. Xii+119pp.

Provasoli, L., J. J. A. McLaughlin and M. R. Droop, 1957: The development of artificial media for marine algae. Arch. Mikrobiol., **25**, 392-428.

Research Department, Fisheries Agency, 1965: Research and study report on extensive aquaculture technique of suitable organisms in coastal area (*Porphyra* sp.) subsidized by the central government, 1962-1964 fiscal year, 90-91. (In Japanese).

Research Department, Fisheries Agency, 1967: Research and study report on extensive aquaculture technique of suitable organisms in coastal area (*Porphyra* sp.) subsidized by the central government, 1965-1966 fiscal year, 6-7. (In Japanese).

Saga Prefecture Aquaculture Experiment Station, 1969: Research and study report on the prevention of industrial damage in the farming *Porphyra* subsidized by the central government. 1968 fiscal year, 1-24. (In Japanese).

Saito, Y., T. Matsusato and K. Yoshikawa, 1972: On the symptoms of "Green spot" and "Grape" in Nori (Porphyra) culture. 5, 1-10. (In Japanese).

Sasaki, M. and S. Sato, 1969: Composition of medium and cultural temperature of *Pythium* sp., A pathogenic fungus, of the "Akagusare" disease of cultivated *Porphyra*. Bull. Tohoku Reg. Fish. Res. Lab., **29**, 125-132. (In Japanese).

SASAKI, M. and Y. SAKURAI, 1972: Comparative observations on the growth among the five strains in *Pythium porphyrae* under the same cultural condition. Tohoku Reg. Fish. Res. Lab., **32**, 83-87. (In Japanese).

SHEWAN, J. M., G. HOBBS and W. HODGKISS, 1960b: The *Pseudomonas* and *Achromobacter* groups of bacteria in the spoilage of marine white fish. J. appl. Bacteriol., **23**, 463-468.

SIEBURTH, J. M., 1975: Microbial seascapes, a pictorials from marine microorganisms and their environment. University Park Press, Baltimore/London/Tokyo, 216pp.

SIEBURTH, J. M., 1968: The influence of algal antibiosis on the ecology of marine microorganisms. In "Advances in microbiology of the sea". ed. M. R. DROOP and E. J. F. WOOD, Academic Press Inc. New York and London. 63-94.

SIEBURTH, J. M., 1969: Studies on algal substances in the sea. III. The production of extracellular organic matter by littoral marine algae. J. exp. mar. Biol. Ecol., **3**, 290-309.

SOLI, G., 1964: A system for isolating phytoplankton organisms in unialgal and bacteria-free culture. Limnol. Oceanogr., **9**, 265-268.

STEIN, J. R., 1973: Handbook of phycological methods. Culture methods and growth measurements. Cambridge University Press. London, xii+448pp.

SUTO, S. and O. UMEBAYASHI, 1954: On the "perforating disease" in nori (*Porphyra*) culture. Bull. Japan. Soc. Sci. Fish., **19**(12), 1176-1178. (In Japanese).

SUTO S., 1960: On the method of a laboratory culture of *Porphyra tenera*. The Aquaculture, 7(3), 7-11. (In Japanese).

SUTO, S., Y. SAITO, K. AKIYAMA and O. UMEBAYASHI, 1972: Text book of diseases and their symptoms in *Porphyra*. Bull. Tokai Reg. Fish. Res. Lab., **18**, e, 1-37. (In Japanese).

TAKAHASHI, M., 1970: Identification method for *Pythium*. Shokubutsu Boeki, **24**(8), 29-36. (In Japanese).

TAKAHASHI, M., T. ICHITANI and T. OKUMURA. 1970: Idenfication and ecology on the pathogen of red rot of *Porphyra*. Bull. Agriculture, Forestry and Fisheries Research Council. 33-46. (In Japanese).

TAMURA, S., 1956: On the bud blight of *Porphyra* in Chiba Prefecture. The Aquaculture, **4**, 44-54. (In Japanese).

TANAKA, N., M. NAKANISHI and H. KADOTA, 1974: The excretion of photosynthetic product by natural phytoplankton in lake Biwa. Jap. J. Limnol. **35**, 91-98.

TANIGUCHI, M., 1970: Studies on the yellow spot disease of conchocelis-I. Influence of light on the attack of the disease. Bull. Japan. Soc. Sci. Fish., **36**(7), 686-691. (In Japanese).

TANIGUCHI, M., 1977a: Studies on the yellow spot disease in conchocelis-II. Method for obtaining the pathogen complex. Bull. Japan. Soc. Sci. Fish., **43**(3), 255-258. (In Japanese).

TANIGUCHI, M., 1977b: Studies on the yellow spot disease in conchocelis-III. Aquatic factors

influencing the development of the yellow spot disease. Bull. Japan. Soc. Sci. Fish., **43**(3), 259-263. (In Japanese).

TATEWAKI, M. and L. PROVASOLI, 1963: Vitamin requirements of three species of *Antithamnion*. Bot. Mar., **6**, 193-203.

TOKUDA, H., 1966: The spread agar plate method for isolation and purification of marine phytoplankton. Bull. Japan. Soc. Sci. Fish., **32**(7), 568-572.

TOMINAGA, T. 1973: A bacterial flora of *Porphyra* sampled in the farming ground. Research studies on the etiological causes of white blight and other *Porphyra* diseases. Agriculture, Forestry and Fisheries Research Council, 58-70. (In Japanese).

TSUKIDATE, J., 1968: A method of measurement of photosynthesis by carbon-14 assimilation. Bull. Tokai Reg. Fish. Res. Lab., **55**, 271-274. (In Japanese).

TSUKIDATE, J., 1970a: Microbiological studies of *Porphyra* plants-I. Studies of bacteria isolation methods. Bull. Nansei Reg. Fish. Res. Lab.., **3**, 19-22.

TSUKIDATE, J., 1970b: Reports on *Porphyra leucosticta* Thuret. Bull. Japan. Soc. Phycol., **18**(3), 164-166. (In Japanese).

TSUKIDATE, J., 1970c: Some notes on *Grinnellia americana* in culture. Bull. Japan. Soc. Sci. Fish., **36**(11), 1109-1114.

TSUKIDATE, J. and S. SUTO, 1971a: Tracer experiments on the effect of micronutrients on the growth of *Porphyra* plant-I. Iron-59 assimilation in relation to environmental factors. Bull. Tokai Reg. Fish. Res. Lab., **64**, 89-100.

TSUKIDATE, J., 1971b: Microbiological studies of *Porphyra* plants-II. Bacteria isolated from *Porphyra leucosticta* in culture. Bull. Japan. Soc. Sci. Fish., **37**(5), 376-379.

TSUKIDATE, J., 1971c: Microbiological studies of *Porphyra* plants-III. Abnormality on the bacterial flora accompanying the plant. Bull. Nansei Reg. Fish. Res. Lab., **4**, 1-12.

TSUKIDATE, J., 1973a: Microenvironment. A disease of *Porphyra*. Fisheries series 2. ed. Japan. Soc. Sci. Fish., 105-113. (In Japanese).

TSUKIDATE, J., 1973b: On the relation between the growth of the *Porphyra* plants cultivated in the sea and the bacterial flora accompanying the plants. Research studies on the etiological causes of white blight and other *Porphyra* diseases. Agriculture, Forestry and Fisheries Research Council, 142-159. (In Japanese).

TSUKIDATE, J., 1974a: Microbiological studies of *Porphyra* plants-IV. On the relation between the growth of the *Porphyra* plants cultivated in the sea and the bacterial flora accompanying the plants. Bull. Nansei Reg. Fish. Res. Lab., **7**, 1-8.

TSUKIDATE, J., 1974b: Tracer experiments on the effect of micronutrients on the growth of *Porphyra* plants-II. Manganese-54 and zinc-65 assimilation in relation to environmental factors. Bull. Nansei Reg. Fish. Res. Lab., **7**, 9-18.

TSUKIDATE, J., 1977a: Microbiological studies of *Porphyra* plants-V. On the relation between bacteria and *Porphyra* diseases. Bull. Nansei Reg. Fish. Res. Lab., **10,** 101-112.

TSUKIDATE, J., 1977b: Microbiological studies of *Porphyra* plants-IV. An investigation of bacteria-free culture of *Porphyra* with a shaking culture apparatus. Bull. Nansei Reg. Fish. Res. Lab., **10,** 113-121.

WATANABE, T., S. KATO, K. ABE, S. NARUSAWA and K. SUZUKI, 1968: Studies on the initiation mechanisms of white wasting disease in the farming ground in Miyagi Prefecture. Bull. Miyagi Prefecure Fisheries Experiment Station, **4,** 53-64. (In Japanese).

WATANABE, T. and S. KATO, 1970: Studies on the disease of cultural *Porphyra*-I. On the nitrogen metabolism in *Porphyra* frond tissue infected with white wasting like disease. Bull. Japan. Soc. Sci. Fish., **36**(9), 921-925. (In Japanese).

WATANABE, T. and S. KATO, 1972: Studies on the diseases of cultural *Porphyra*-IV. On the cause of cancer disease. Bull. Japan. Soc. Sci. Fish., **38**(5), 431-437. (In gapanese).

WILLIAMS, J. L., T. BERMAN and O. Holm-HANSEN, 1976: Amino acid uptake and respiration by marine heterotrophs. Mar. Biol., **35,** 41-47.

YAMADA, H., A. MURAKAMI and M. KAYAMA, 1979: Mineralization of organic substances in sea water. Bull. Japan. Soc. Sci. Fish., **45**(10). 1299-1305.

YAMAGUCHI PREFECTURAL NAIKAI FISHERY EXPERIMENTAL STATION, 1972: Research and study report on *Porphyra* disease subsidized by the central government, 1971, fiscal year, 1-55. (In Japanese).

YAMASHITA, T., 1969: Studies on the initiation mechanisms of white wasting diseases-II. Bull. Fukuoka Prefecture Ariake Fisheries Experiment Station, 1, 1-27. (In Japanese).

YAMAZAKI, H., Y. FURUIDO and S. ITO, 1956: On the causes and measurements of poor production in *Porphyra* farming in Tokyo districts, 1-13. (In Japanese).

ZoBELL, C. E., 1946: Marine microbiology. Chronica Botanica Co. Waltham. Mass. 240pp.

Explanation of Plates I—III

Plate I

Red cells found in the thalli grown on the rocks or the nets in the sea.

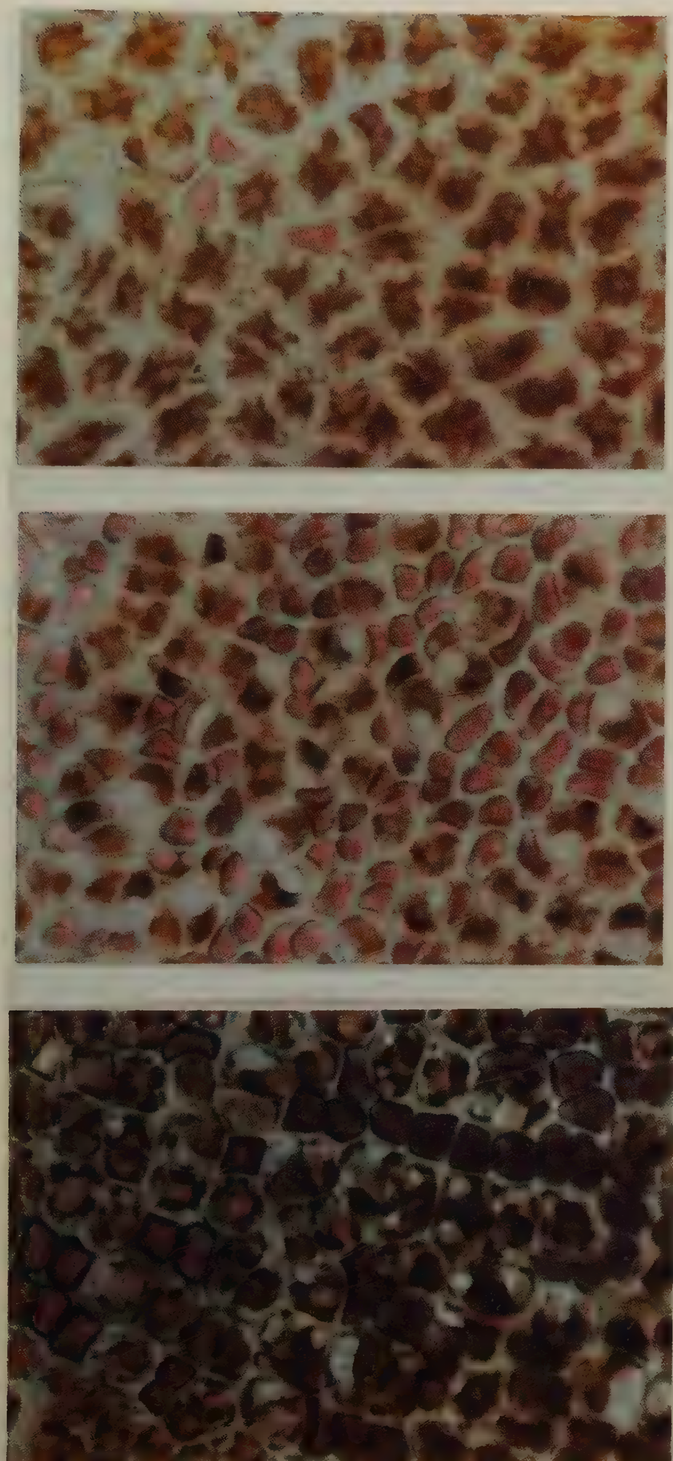


Plate II

White cells found in the thalli grown on the rocks or the nets in the sea.

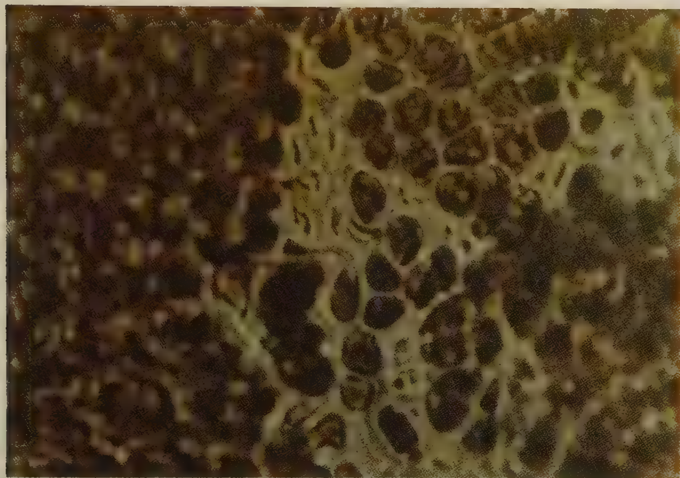
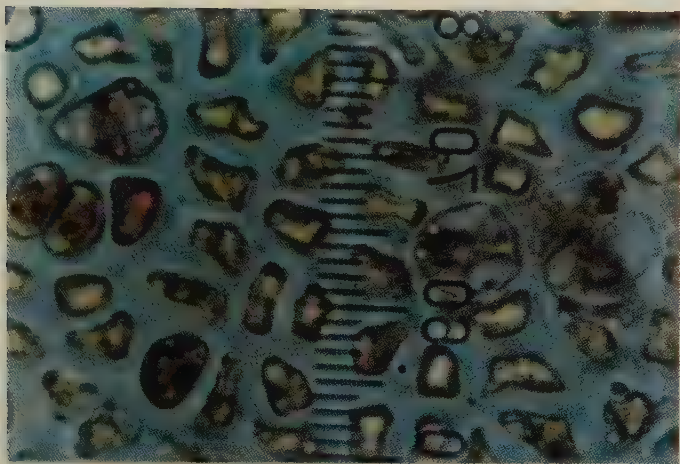


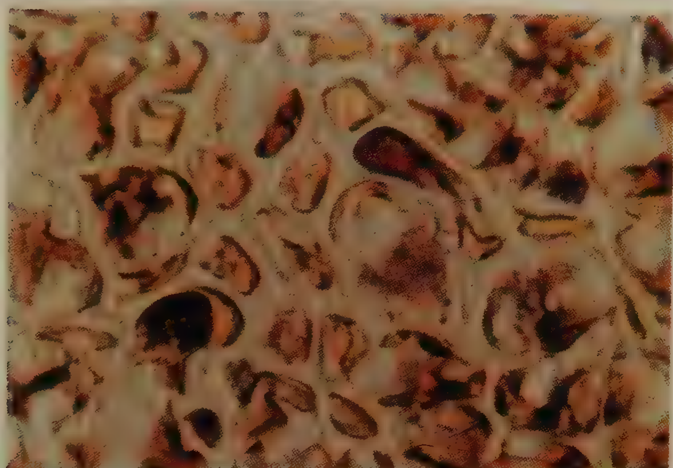




Plate III

Red and white cells caused with the inoculation of *Beneckea* sp..







Symbiotic relationship, bacterial pathogen

ノリと着生細菌の相互作用及び白ぐされ病原因菌について

月　舘　潤　一

本研究は，わが国において養殖ノリに発生する病害のうち，産業的被害の最も大きい「白ぐされ病」の原因を調べるために行われた。その結果，ノリと細菌との間に相互関係の存在することが明らかとなり，又，細菌が本病の原因に関与していることも明らかとなった。これにより，原因菌が判明し，病気発生の機構が明らかになり，養殖の過程で生理異状の起きないように，養殖技術を適正に管理するなどの防除対策を立てる基礎資料を得た。本研究で得られた結論は以下のとおりである。

1)　ノリ葉体の表層には，粘液層があり，その中に無数の細菌が存在することから，まず健全なノリ葉体上の菌叢を調べたところ，実験室で培養しているノリでは *Micrococcus*, *Achromobacter*, *Flavobacterium*/*Cytophaga*, *Pseudomonas* から成り，このうち *Achromobacter*, *Flavobacterium*/*Cytophaga*, *Pseudomonas* が常時みられた。又，天然の岩に自生しているノリ及び，養殖しているノリからは，*Micrococcus*, *Achromobacter*, *Flavobacterium*/*Cytophaga*, *Pseudomonas*, *Aeromonas* 及び yeast が分離され，このうち *Achromobacter*, *Flavobacterium*/*Cytophaga*, *Pseudomonas* が常時存在していた。従って，*Achromobacter*, *Flavobacterium*/*Cytophaga*, *Pseudomonas* がノリの着生細菌と考えられる。

2)　1973，1974，1975年にわたるノリ養殖年度において，ノリ病害調査を行なった。ノリには，前述のごとく着生細菌が存在するので，病葉だけでなく，健全葉体から病葉体になるまでの，全過程を通じて，定期的に葉体を調べ，細菌の分離を行った。その結果，いずれの年度においても，ノリ葉体上で *Beneckea* 属の細菌が菌叢の大部分を占めると，ノリの細胞は白色化して死滅するものが増加し，症状が進むと葉体の流失が見られた。

この *Beneckea* 属の細菌は，1973，1974，及び1975年のいずれの年度の分離菌株も諸性状が調べられた範囲では一致しており，これらの性状は均一で同一種と推定された。又，Type species の *Beneckea campbellii* ATCC-25920とは，かなり類似していたが，サッカローズ，ラクトーズ，アラビノーズ，キシローズの分解活性が異なり，TMAO，リジンの脱炭酸，10％食塩加培地での発育能，40℃における発育などの点で明らかに異なり *Beneckea campbellii* とは同定し得なかった。

更に Baumann ら（1971）及び Lee ら（1978）の *Beneckea* 属菌株とくらべても性状は細部で異なり，既報のいずれの菌株とも一致しないため *Beneckea* sp. とした。

3)　*Beneckea* sp. の病原性を調べるため，室内実験で，発症実験を行なった。*Beneckea* sp. を接種すると，ノリ葉体はすべて「白ぐされ病」の病徴を呈し，葉体は崩壊して原形が失われ，ついには消失するに致った。その際，病葉体から *Beneckea* sp. の再分離を試みたが，5回のうち4回は出現率が100％であり，残り1回は95％であった。このように，*Beneckea* sp. の病原性が明らかとなり，「白ぐされ病」の原因菌のひとつであることが確認された。

4)　ノリと着生細菌の相互関係を明らかにするために *Beneckea* sp. 及び健全なノリ葉体から分離された細菌の形態的，生物学的，生化学的性状を調べた。その結果，*Beneckea* sp. は好塩菌であり，20℃でよく増殖し，着生細菌の *Achromobacter*, *Flavobacterium*/*Cytophaga*, *Pseudomonas* と比較して，アミノ酸に対する要求が異なっており，特に，アスパラギン酸，グルタミン酸を利用することが判明した。

5)　着生細菌はアミノ酸を利用して増殖するが，その結果，炭酸ガスを放出することが推定され，放射性物質のアミノ酸を用いて調べた。その結果，供試した7株の細菌は *Beneckea* sp. も含めて，すべてアミノ酸を利用し，分解して炭酸ガスを放出したが，細菌の種類により利用するアミノ酸が異なり炭酸ガスの放出量も異なった。そして，*Beneckea* sp. は，プロリン，グルタミン酸，アスパラギン酸，アラニン，セリンをよく分解した。

6)　藻類は代謝産物を細胞外に放出することが知られているが，ノリも生長の過程で，有機物質を細胞外に放出することを，放射性同位元素の炭素を用いて明らかにした。更にノリの着生細菌は，アミノ酸を利用し増殖しているという前述の結果から，ノリを培養した培養液中のアミノ酸を分析したところ，ノリは生長の過程で16種類のアミノ酸を細胞外に放出することが判明した。しかも水温をあげると，放出されるアミノ酸の組成には変化はないが，放出量が変り，アスパラギン酸，グルタミン酸，グリシン，ロイシン，チロシン，フェニールアラニン，

ヒスティディン，リジン，アルギニンが多くなることが明らかとなった。

7) 有機物質の欠乏は生理異状の原因となる。又，着生細菌はアミノ酸を利用して，炭酸ガスを生成しているので，ノリを無菌にして各種栄養塩の吸収を調べ，炭酸の影響も調べた。そこで，無菌培養を行ない，各種の有機物質を培養液に添加したが，生長はよくなかった。しかし，炭酸量を増加して培養したところ，生長が混菌培養と同程度になり，無菌ではノリの生長に炭酸ガスの補給が必要であることを明らかにした。このことから着生細菌はノリに炭酸ガスを補給していることが確認された。

8) 以上の諸結果から，ノリと着生細菌との間には次のような関係のあることが考えられる。即ち，ノリは着生細菌にアミノ酸を補給し，着生細菌はこのアミノ酸を利用して増殖し，その際，細菌は炭酸ガスを放出してノリの生長を促進する。又，細菌性の「白ぐされ病」が，発生する機構は以下のように考えられる。ノリと着生細菌との関係が，気象，海況の諸条件の変化で乱れて，ノリの放出するアミノ酸の量が異なり，アスパラギン酸及びグルタミン酸が増加すると，***Beneckea*** **sp.** が出現し，「白ぐされ病」が発生する。

南西水研報
Bull. Nansei Reg. Fish.
Res. Lab. No. 15, 1983

Effect of Prey Density on the Swimming Behaviour of Larval Black Porgy, *Acanthopagrus schlegeli* (BLEEKER)

Osamu FUKUHARA

Abstract

The swimming behaviour of larval black porgy, *Acanthopagrus schlegeli* (BLEEKER) was investigated, with particular attention to prey density under the laboratory conditions. Cultured rotifer, *Brachionus plicatilis*, and *Artemia* nauplii were employed as food organisms. Observations on swimming speed were made on 14, 16, 21, 22 and 26 days after hatching. No significant correlation was found between swimming speed and prey density for 14-day-old larvae. But older larvae swam very slowly just after they were fully fed, and the fish became more active together with decreasing prey density. No decrease of swimming speed occurred at a prey density of more than five rotifers/m*l* for 14, 16, 21 days old larvae.

Consequently, the swimming activity of larval black porgy are related closely with prey conditions in the tank.

Suitable prey density is essential for survival of larval fish during rearing as well as in the wild. Shortage of food may cause high mortalities in a rearing tank and may cause a decrease of natural populations. On the other hand, larval fish also have some tactics for survival under unsuitable environmental conditions. Knowledge of the feeding behaviour of larvae is prerequisite for rearing and clarifying mortality in the wild.

Larval behaviour in relation to feeding was reviewed by HUNTER (1980, 1981). But little is known about feeding behaviour in relation to prey density during rearing. In this paper, I describe the feeding behaviour of larval *Acanthopagrus schlegeli* during rearing in the laboratory.

Materials and Methods

Larval Rearing

Eggs from cage-cultured fish were collected at the Hiroshima Prefectural Fisheries Experimental Station on June 15, 1981. Eggs were transported to the Nansei Regional Fisheries

Received: November 25, 1982. Contribution No. 129 from the Nansei Regional Fisheries Research Laboratory, Ohno-cho, Hiroshima-Ken, 739-04, Japan

Research Laboratory and incubated at 19°C. Hatching occurred on June 17. Larvae were reared until August 16 (60 days).

Rearing methods were similar to those described by FUKUHARA (1977). The rectangular, plastic (FRP) rearing tank measured 250×95×70 cm depth.

Mean water temperature was 23.3°C, ranging from 19.4° to 25.7°C; mean salinity was 29.8‰, ranging from 27.5 to 31.4‰, and mean illumination was 1890 lux, ranging from 540 to 5000 lux at the surface of the rearing tank in the morning (8:30-9:00).

Observing and Estimating Swimming Behaviour

Observations on swimming behaviour were made 14, 16, 21, 22, and 26 days after hatching. Devices used for observing larval swimming were the same as used for studies of *Pagrus major* (FUKUHARA and KISHIDA 1980). Swimming paths were traced for ten larvae, chosen at random, before and after feeding. It took about 20 minutes to observe ten larvae. Swimming speeds were calculated from the swimming paths using a graphic tablet (Hewlett-Packard, 9111A).

Brachionus plicatilis and *Artemia salina* were used as food organisms. Prey density was determined from three counts of one-m*l* sample.

No experiments were carried out on larvae younger than 14 days or older than 26 days because of difficulty with varying the prey density adequately. Larvae younger than 14 days were not able to reduce the prey density sufficiently during the feeding period, while larvae older than 26 days (nearly at the transition stage from post-larvae to juveniles) did not feed well because the prey was too small.

Results and Discussion

Fig. 1 shows the growth of larvae reared in the experiment. Daily growth rate in this experiment was similar to that in 1977 (FUKUHARA 1977); 0.20 mm for the former and 0.21 mm for the latter. Therefore, larval growth shown in Fig. 1 was recognized as a usual development of porgy reared in the laboratory conditions.

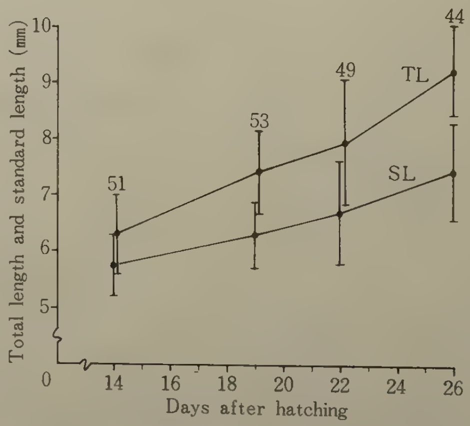


Fig. 1 *Acanthopagrus schlegeli*. Growth of larvae during rearing. Each measurement indicates averages, standard deviation and sample size.

Swimming speeds of larvae increased with decreasing prey density at each age (Fig.2). For 14-day-old in the relation was not as good as observed in the other groups. Swimming speeds ranged from 3 to 4 mm/sec. just after feeding, except for 14-day-old larvae, and then with a

decrease of food density swimming speeds increased gradually to more than 10 mm/sec. which were observed before feeding. Variations in swimming speeds were less just after feeding than when prey density was low. It is assumed that larval fish overcome the shortage of food organisms by increasing searching area. FUKUHARA and KISHIDA (1980) observed the

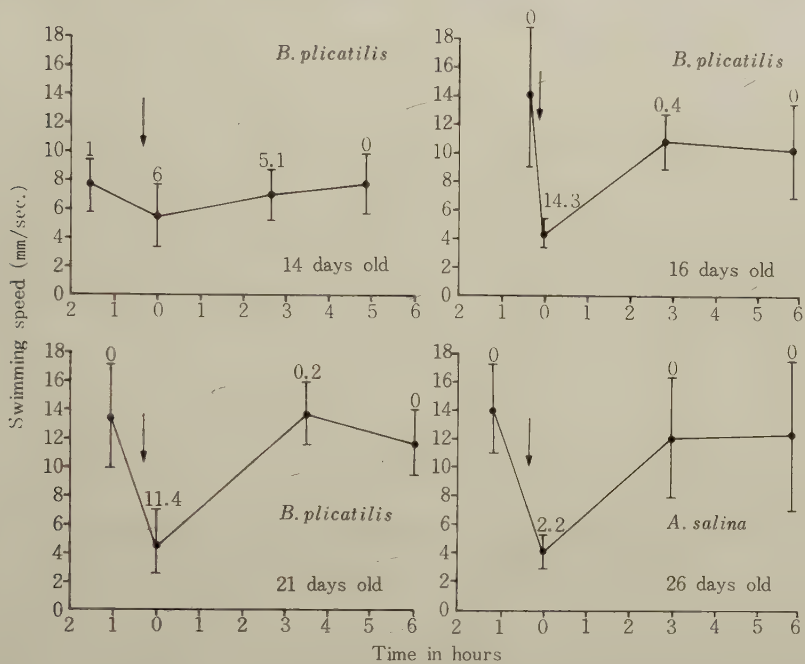


Fig. 2 *Acanthopagrus schelegeli*. Changes of swimming speed related with prey density. Arrows indicate feeding, and numbers at the times of measurement (mean ± SD) indicate prey density per m*l*.

swimming speed of red sea bream larvae at different food densities by using same device (pantagraph) described herein. According to their experiments, younger larvae showed no clear difference in swimming speed before and after feeding; however, older larvae did show such a difference. Consequently, it appears that the swimming speed of the larvae was affected by prey density and larval age.

Fig. 3 shows the relationship between swimming speed and food density. Swimming speeds greater than 10 mm/sec. were recorded, except for 14-day-old larvae, when no food organisms were present. A decrease of swimming speed was found with increasing prey density to the level of 5 rotifers /m*l*, and then no decrease was observed when prey density was greater than 5 individuals/m*l*.

Swimming speed of larval fish is important for searching for food organisms as well as for escaping from predators. At lower prey densities, larval plaice (WYATT 1972) and anchovy (HUNTER and THOMAS 1974) swam faster than that at higher ones. This tendency was more

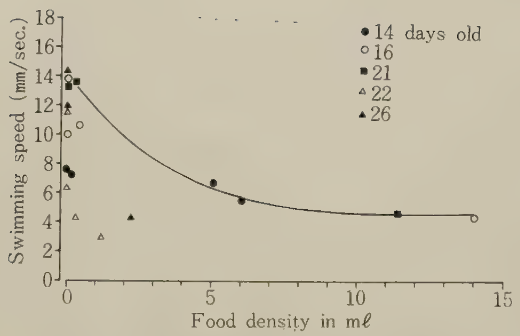


Fig. 3 *Acanthopagrus schlegeli*. Relatioship between swimming speed and prey density for larvae of various ages. Each plot shows the average of ten observations. *Brachionus plicatilis* was added from 14 to 21 days after hatching, and *Artemia salina* after 26 days. Curve was drawn freehand.

pronounced for older fish than for younger. It indicates that during rearing, younger larvae must be maintained with higher prey densities than that older ones.

In this study, no increase of swimming speed occurred at prey densities greater than 5 rotifers /m*l*. These findings suggest there is an optimum density for obtaining high survival of larvae.

I would like to extend my thanks to Dr. Alan W. White for careful reading of the manuscript. Thanks are also due to Mr. Satoshi Umezawa for suggesting to analysis the data.

References

FUKUHARA, O. 1977: Some morphological observations on larvae and juveniles of the Kurodai, *Mylio macrocephalus* (Sparidae: Teleostei) reared in the laboratory. Bull. Nansei Reg. Fish. Res. Lab., (10), 1-16.

FUKUHARA, O. and T. KISHIDA, 1980: Some observations on the swimming behaviour as related to feeding in the red sea bream larvae reared under laboratory conditions. Bull. Nansei Reg. Fish. Res. Lab., (12), 9-20.

HUNTER, J. R. and G. L. THOMAS, 1974: Effect of prey distribution and density on the searching and feeding behaviour of larval anchovy *Engraulis mordax* GIRARD. In "The early life history of fish" ed. J. H. S. BLAXTER, Springer-Verlag Berlin Heidelberg, 559-574.

HUNTER, J. R. 1980: The feeding behavior and ecology of marine fish larvae. In "Fish behavior and its use in the capture and culture of fishes". ed. J. E. BARDACK, J. J. MAGNUSON, R. C. MAY and J. M. REINHART, ICLARM, Manila, 287-330.

HUNTER, J. R. 1981: Feeding ecology and predation of marine fish larvae. In "Marine fish larvae". ed. R. LASKER, Washington Sea Grant Program, Washington, 33-77.

WYATT, T. 1972: Some effects of food density on the growth and behaviour of plaice larvae. Marine Biology, 14, 210-216.

クロダイ仔魚の遊泳に及ぼす餌密度の影響

福　原　　　修

クロダイ仔魚の飼育条件下における摂餌行動と餌料密度との関係を明らかにするため，ふ化後14，16，21，22および26日の仔魚に生物餌料（シオミズツボワムシ，アルテミア幼生）を与え餌料密度と遊泳速度の変化を観察した。

ふ化後14日目の仔魚は，餌料密度と遊泳速度の間に顕著な相関はみられなかった。ふ化後14日目を除く仔魚においては，給餌前の遊泳速度は速く，摂食直後は最も遅くなった。そして仔魚の摂取による餌料の減少に伴って 4 ㎜/sec. 前後であった速度は次第に増加し，給餌前の10〜12 ㎜/sec. になった。また，14〜21日目の仔魚にシオミズツボワムシを与えた場合，5個体/ml 以上の密度では速度の変化はみられなかった。

これらのことからクロダイ仔魚の遊泳速度と餌料密度は密接な関係を有し，餌料が不足した場合は遊泳速度の増加で索餌範囲を拡大していること，またある密度までは餌料の増加に伴い速度が遅くなると推察された。

南西水研報
Bull. Nansei Reg. Fish.
Res. Lab. No. 15, 1983

薩南海域におけるマイワシの卵・仔魚の分布，1976—1981年

小 西 芳 信

Distribution of Eggs and Larvae of Sardine in the Satsunan Area, 1976-1981

Yoshinobu KONISHI

Japanese sardine, *Sardinops melanosticta* (TEMMINCK and SCHLEGEL), represents one of the most widely fluctuating species. Drastic changes were well documented not only in amount of catch but also in geographic distribution of the species for the half a century since the 1920's (NAKAI 1962, WATANABE 1981). A feature of the species population found in the past prosperous years of the 1930's was dominancy of spawning in the Satsunan Area that produced about 70% of total eggs estimated by the census.

After gradual recovery of the species population in recent years, significant spawning occurred in the Satsunan Area since 1976. The author had an opportunity to investigate the expansion of distribution range of eggs and larvae of sardines in conjunction with the increase of spawners based on regular plankton net collection and related surveys.

In the recent six years from 1976 to 1981, spawning sardines were abundantly located in the coastal waters along the Satsuma Peninsula and the Oosumi Peninsula (Long. 129°40′E to 131°40′E), the Oosumi Strait and the waters surrounding the Yakushima and Tanegashima Islands. The distribution range was restricted in the coastal waters from the Satsuma Peninsula to the Oosumi Peninsula in 1976, while extended year after year southward since then. In and after 1979, the spawners gathered in the waters surrounding the Yakushima and Tanegashima (Isls). The fish appears on the increase in the size of stocks.

The expansion of the distribution range of the spawning sardines in the Satsunan Area accompanied change of occurrence of eggs and larvae. In the early three years from 1976 to 1978 eggs at very early developmental stage, or A-stage defined by NAKAI (1962), were found distributed in the coastal waters from the Satsuma Peninsula to the Oosumi Strait. Advanced eggs at B- and C-stages and larvae were found in the offshore waters seemingly transported from the coastal waters. In 1979, eggs and larvae decreased in number and were found distributed in the coastal waters from the Satsuma Peninsula to the Oosumi Strait and in the waters surrounding the Yakushima and Tanegashima (Isls). They were found transported

1982年11月25日受理，南西海区水産研究所業績第130号，〒739-04 広島県大野町　住所　南西海区水産研究所高知庁舎，〒780 高知市桟橋通り

eastward. In 1980 and 1981, eggs and larvae occurred in the south waters surrounding the Yakushima and Tanegashima Islands. They were found transported eastward, and some of them drifted beyond the major axis of the Kuroshio Current. In 1981, the eggs and larvae were collected in the almost whole offshore area in the countercurrent.

1930年代の豊漁期には薩南海域で産出されたマイワシ *Sardinops melanosticta*（Temminck and Schlegel）の卵は黒潮によって本州太平洋岸に運ばれていたが，資源が小さくなると産卵場は他の海域に移動した（Nakai 1962)。1970年代に入りマイワシ資源が回復するに及び1976年1，2月に再び顕著な産卵場が薩南海域で形成された。この間卵・仔魚の分布域は黒潮の沿岸側のみでなく，本流および沖合側にも広がっていることがわかった。

一つの海域で消滅した産卵場が再び形成される過程を明らかにすることは，資源の分布量の長期変動を理解する上できわめて重要である。1976年から1981年に薩南海域で産卵場が拡大する過程は，毎年1，2月に調査船俊鷹丸によって行なわれる海洋調査，卵・稚仔調査によって把握されている。本研究では同船の資料に基づく観察結果を述べる。

本報告をとりまとめるにあたって，ご指導とご校閲をいただいた林繁一企画連絡室長に厚くお礼申し上げる。さらに，冬季の厳しい条件下の調査に一緒に乗船し，また貴重なご助言を得た外海資源部第1研究室古藤力室長と流況と魚群分布の資料を提供された同花岡藤雄主任研究官，ならびに図版作成に手を煩した同中沢すみ子技官に心からお礼申し上げる。また，ジェットネットのえい網方法等に関しご指導願った赤潮部安楽正照部長（前西海区水産研究所）と西海区水産研究所木元克則技官，ならびにこの調査を実施するにあたり多くのご協力を頂いた遠洋水産研究所所属俊鷹丸角田精一船長をはじめ乗組員の方々に対し深甚の謝意を表する。

資　　料

南西海区水産研究所外海資源部は，マイワシを含む重要沿岸表層性魚類の発生と補給状態およびそれらを支配する環境条件，とくに黒潮の変動を調べるために，1970年以来引き続いて調査船によって潮岬から屋久島，種子島に至る水域を観測している。今回用いた資料は1，2月に薩南海域およびその周辺で，1976年から1981年の6ヵ年に遠洋水産研究所調査船俊鷹丸（393.44トン）によって行なわれた海洋観測，卵・仔魚採集，魚群探知機による記録の結果である。薩南海域は北緯29°20′—31°25′，東径128°30′—133°00′の範囲とする。

調査定線・定点は魚群の出現状態，黒潮の流況，他の調査との関係を考慮して設定されたので年年異っているが黒潮本流とその周辺をおおっている。(Figs. 1—6)。1980，1981年にはさらに種子島東方から足摺岬沖の黒潮の沖合側でも調査が行なわれた。以下，黒潮本流の沿岸側を黒潮内側域，その沖合側を黒潮外側域と呼ぶ。

各調査定点では一般気象・海象，水温，塩分，GEKによる表層流観測と㋠ネット，㊥ネットによる卵・仔魚の採集を行なった。定点間では魚群探知機により魚群を調査し，1981年にはジェット

ネットによる卵・仔魚採集を加えた。

魚群探知機による調査では魚群存在量の指標として出現した累積距離を用いた。㋠ネットは船速2ノットで表層を5分間水平にひいた。㊥ネットは水面下の150m層から表面まで，ただし水深が150mより浅い場合には海底付近から垂直にひいた。このネットには口部に沪水計を装着した。ジェットネット（TANAKA *et al* 1968, 1969）は，調査定点における調査を修了し調査船が航走を開始した直後に船尾の右舷側から投入し，水面下の5—10m層を船速8—10ノットの高速でつぎの調査定点まで連続的に水平びきした。

採集物はすべてホルマリンで固定し，研究室において選別，同定，算定した。マイワシ卵は NAKAI（1962）に従って初期，中期，後期の発生段階毎に，仔魚は全長階級毎にそれぞれ算定し，内田他（1958）が規定した仔魚前期の最大全長の6㎜未満を小型魚，6㎜以上を大型魚として集計した。1976，1977，1979，1980年の資料は㋠ネット，1978年のそれは㊥ネットによる採集物である。1981年の資料は3種のネットの採集物であり，仔魚は全採集数で示した。また，採集数の多い標本ではその一部を無作為に抽出，算定して全数を推定した。個体数は㋠ネット採集物では1えい網当り，㊥ネット採集物では沪水計回転数とネットの口部面積によって求めた沪水量100㎥当り，ジェットネット採集物では沪水率を100%と仮定して電磁ログの航程指示器から読み取ったえい網距離と口部面積によって求めた沪水量1000㎥当りの値に換算して用いた。

なお，この資料に基づく流況と魚群分布は毎年花岡（未発表）によって整理され漁海況予報に用いられている。

結　　　果

流　況

花岡（未発表）が作成したＧＥＫ測流結果およびこれに基づく1976—1981年の各期の薩南海域における黒潮本流の位置を Fig. 1—6・A に示した。また，Fig. 7 に1981年の薩南海域を含む南西外海の全域における1，2月の流況を示した。

1976年1，2月には屋久島北西で黒潮は薩摩半島に接近した後，南下し屋久島，種子島と都井岬南東で接岸していた。屋久島北西で蛇行していた黒潮の東側ではそれから流出した強勢な南東流が卓越していた。種子島東方の黒潮外側域では弱い南西流がみられた（Fig. 1・A）。

1977年1，2月には黒潮は屋久島西方で観測されず種子島東方をやや離岸して流れていた。薩摩半島南西の沿岸では弱い南西流，志布志湾沖合ではやや弱い南流がそれぞれみられた（Fig. 2・A）。

1978年1，2月には黒潮流路は屋久島の西側で接岸し種子島東方でやや離岸しており，顕著に蛇行していた。屋久島北西の黒潮内側域では強勢な南東流がみられた。大隅海峡ではやや強い東流がみられ，その勢力は種子島の北側を越えて黒潮本流に達していた。南日向灘とその南側では弱い南下流がみられた（Fig. 3・A）。

1979年1，2月には黒潮は屋久島西方では観測されず種子島東方から都井岬東方にかけて離岸していた。屋久島の北西水域ではやや弱い南東流が卓越していた。種子島の東側の黒潮内側域では大

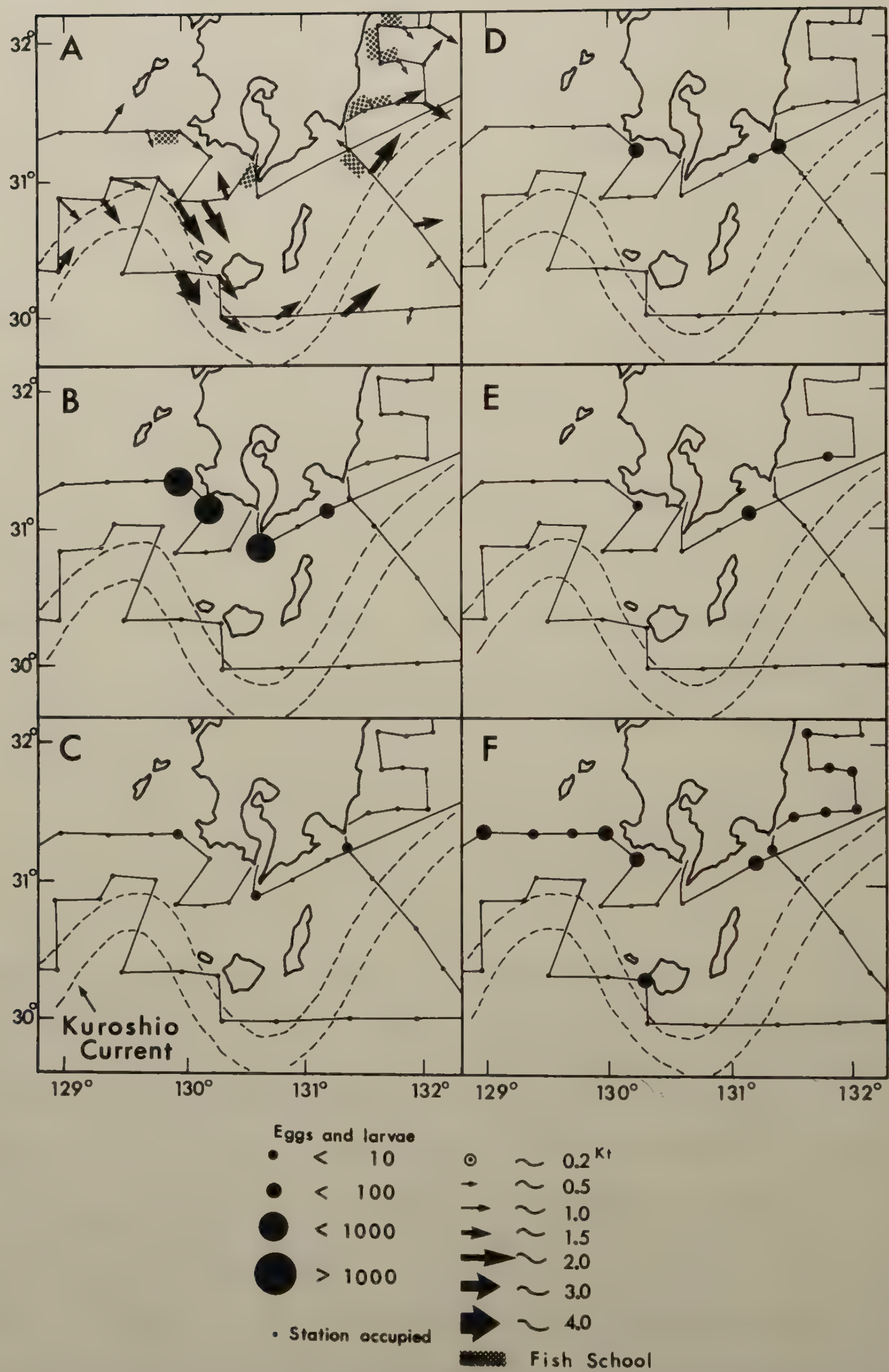


Fig. 1 Distribution of surface water current measured by GEK, shoals of sardine located by echo sounder and eggs and larvae of sardine collected by *Maruchi* net in the Satsunan Area and adjacent waters by R/V SHUNYO MARU during Feb. 9—21, 1976.

A: surface water current and echo trace of sardine (modified from Hanaoka, unpublished), B: eggs at early developmental stage, or A-stage defined by Nakai (1962), C: eggs at middle developmental stage, or B-stage, D: eggs at later developmental stage, or C-stage, E: larvae of 3-6 mm in total length, F: larvae of 6-20 mm in total length.

きな反時計回りの環流がみられ，これに大隅海峡にみられる南東流と南日向灘からの南下流が入り込んでいた（Fig. 4・A）。

1980年1，2月には屋久島の南側を迂回した黒潮は種子島の東側から都井岬の南東にかけて接岸していた。屋久島の北西水域ではやや弱い南西—南東流が卓越していた。種子島から都井岬に至る黒潮内側域では黒潮に向う流れがみられた。黒潮外側域では時計回りの反流が見られた（Fig. 5・A）。

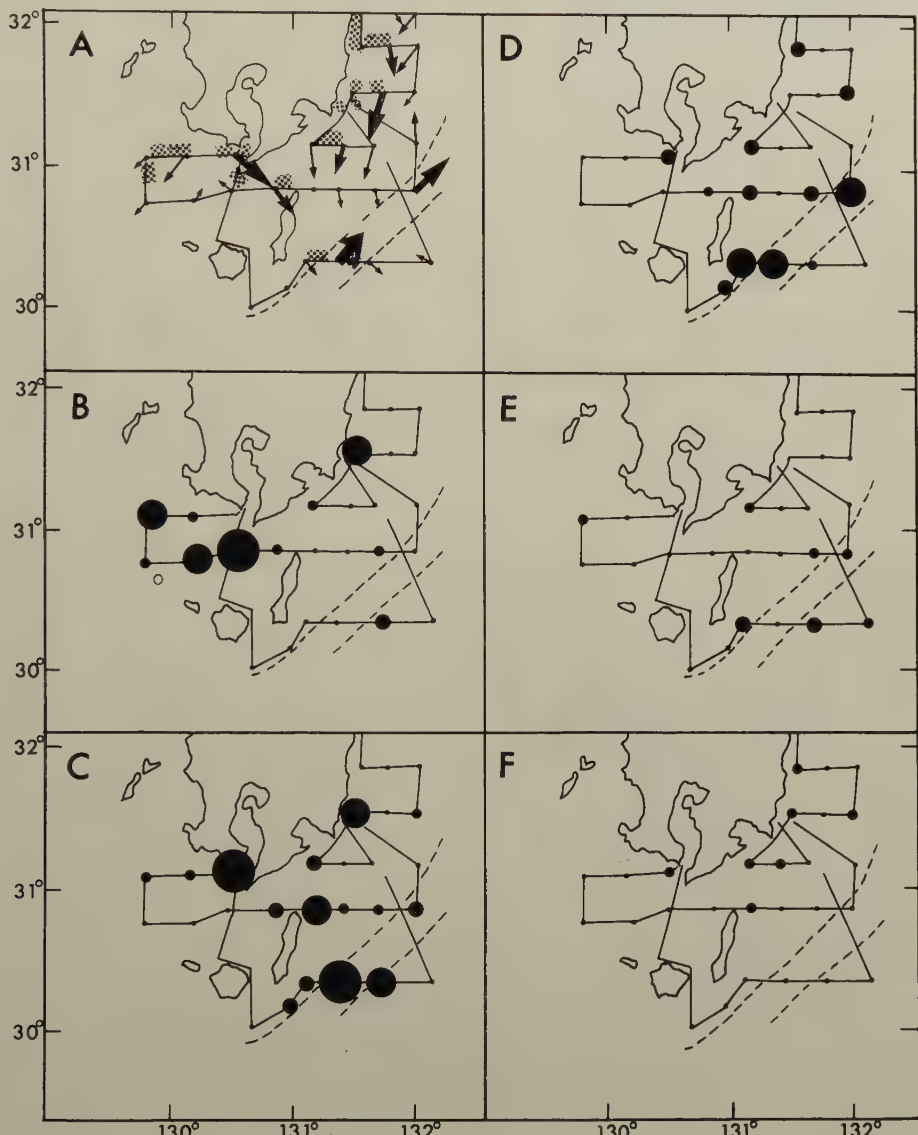


Fig. 2 Distribution of surface water current measured by GEK, shoals of sardine located by echo sounder and eggs and larvae of sardine collected by *Maruchi* net in the Satsunan Area and adjacent waters by R/V SHUNYO MARU during Feb. 9–19, 1977.
See footnote of Fig. 1 for legends.

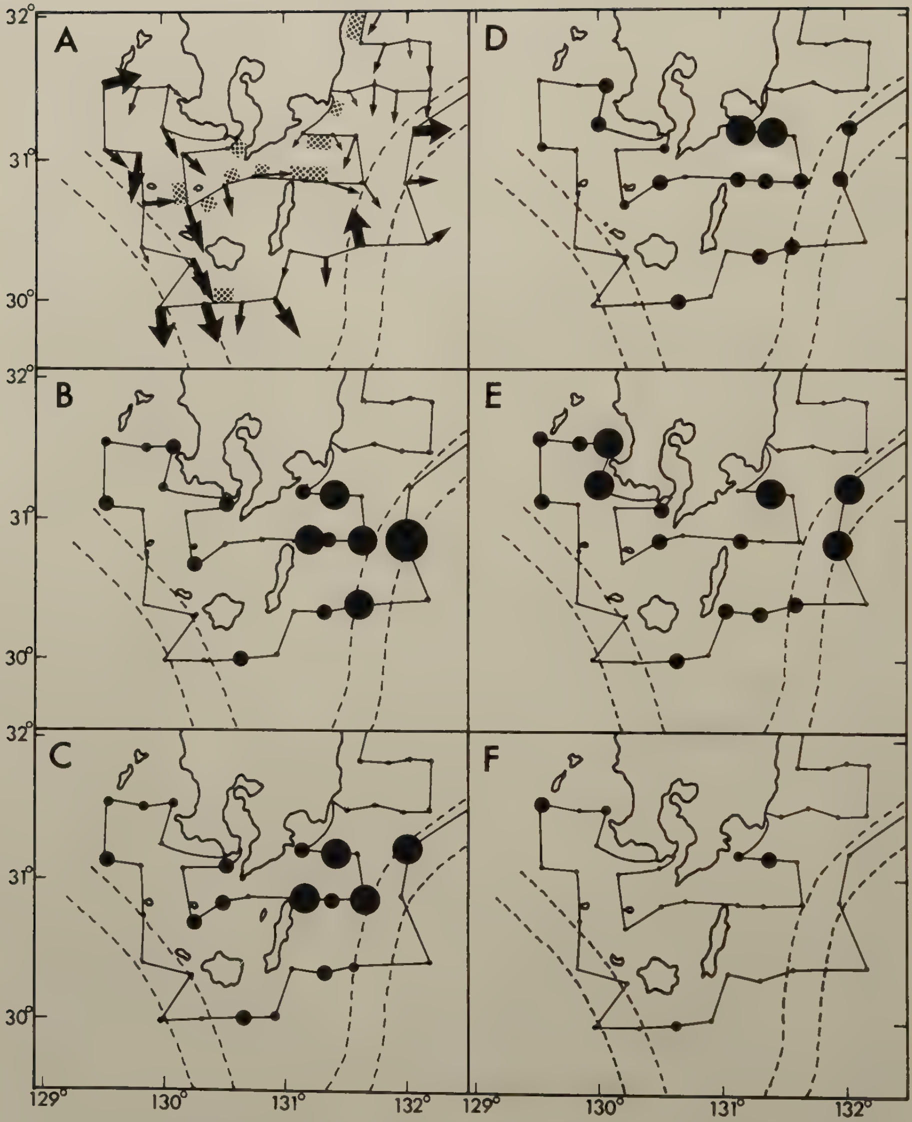


Fig. 3 Distribution of surface water current measured by GEK, shoals of sardine located by echo sounder and eggs and larvae of sardine collected by *Marunaka* net in the Satsunan Area and adjacent waters by R/V SHUNYŌ MARU during Feb. 1–8, 1978.
See footnote of Fig. 1 for legends.

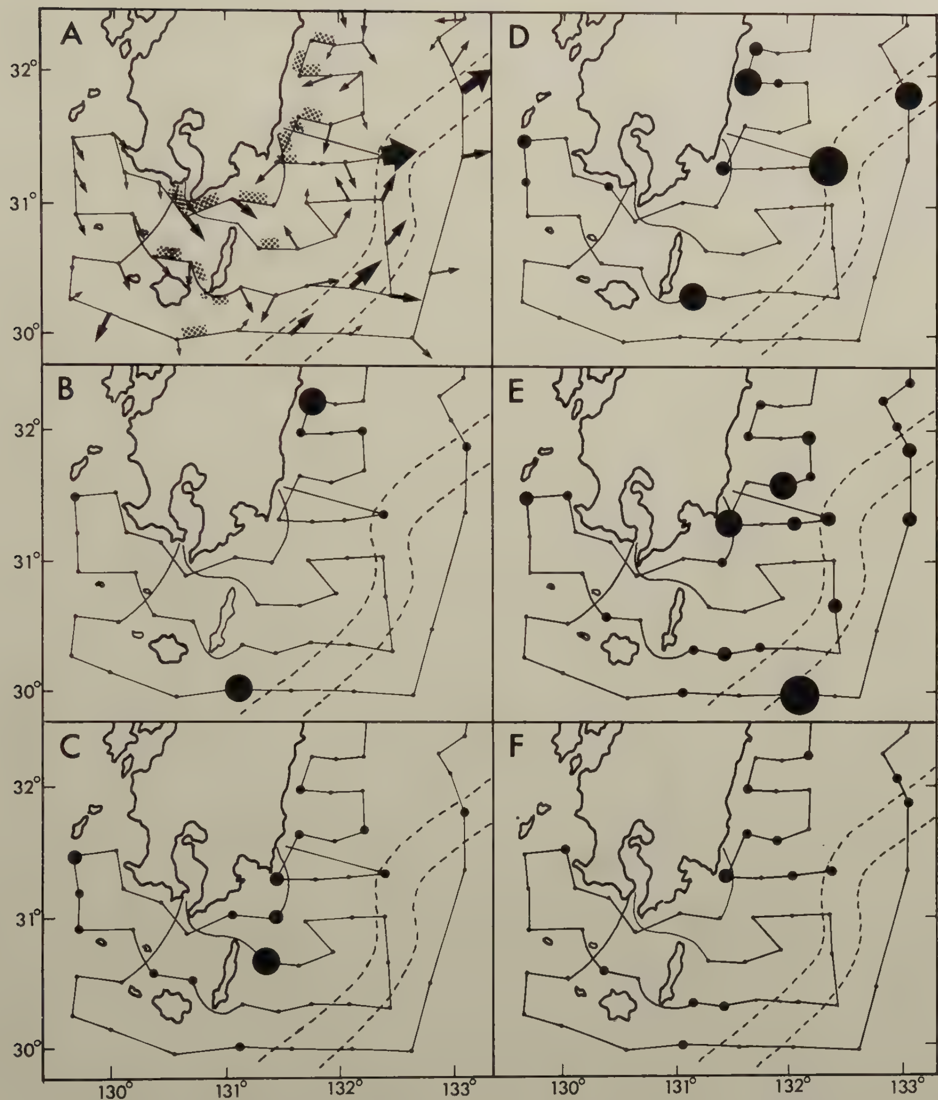


Fig. 4 Distribution of surface water current measured by GEK, shoals of sardine located by echo sounder and eggs and larvae of sardine collected by *Maruchi* net in the Satsunan Area and adjacent waters by R/V SHUNYŌ MARU during Feb. 2–12, 1979.
See footnote of Fig. 1 for legends.

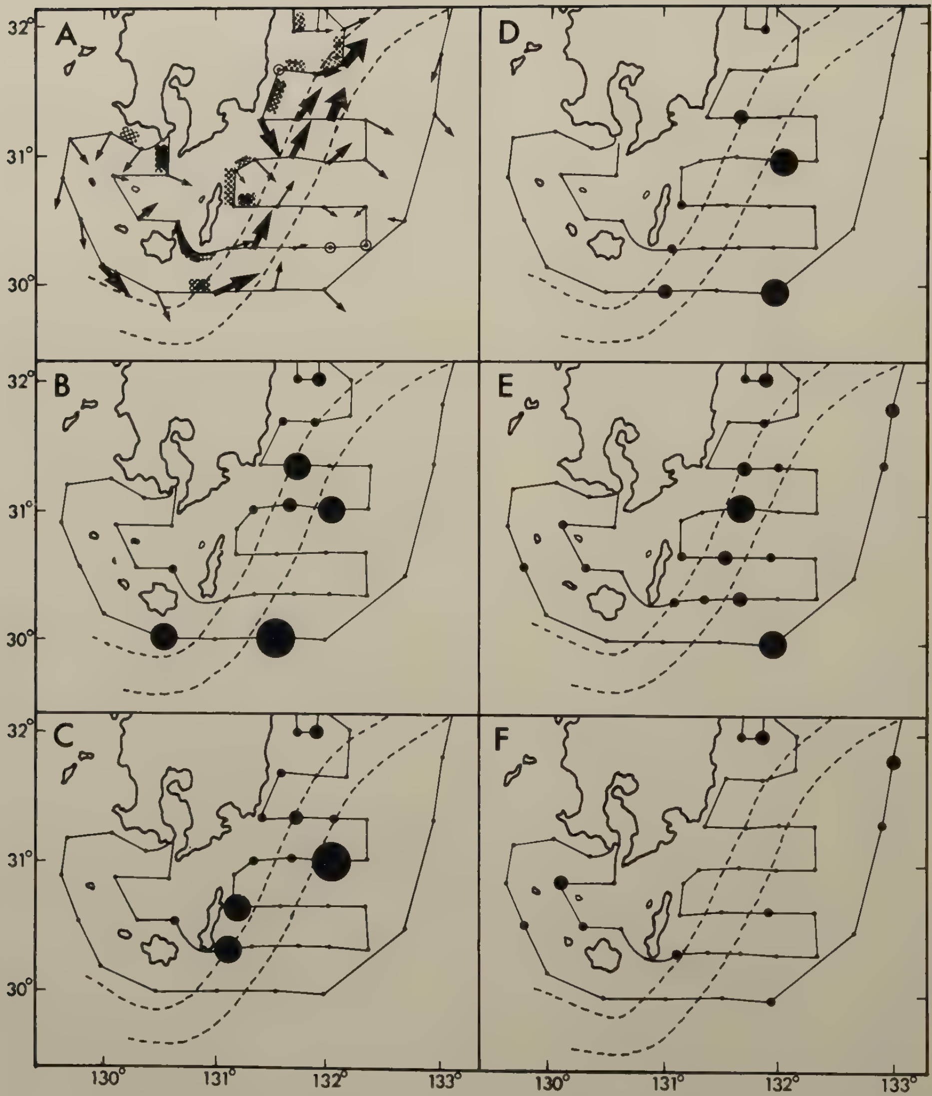


Fig. 5 Distribution of surface water current measured by GEK, shoals of sardine located by echo sounder and eggs and larvae of sardine collected by *Maruchi* net in the Satsunan Area and adjacent waters by R/V SHUNYŌ MARU during Feb. 6-15, 1980.
See footnote of Fig. 1 for legends.

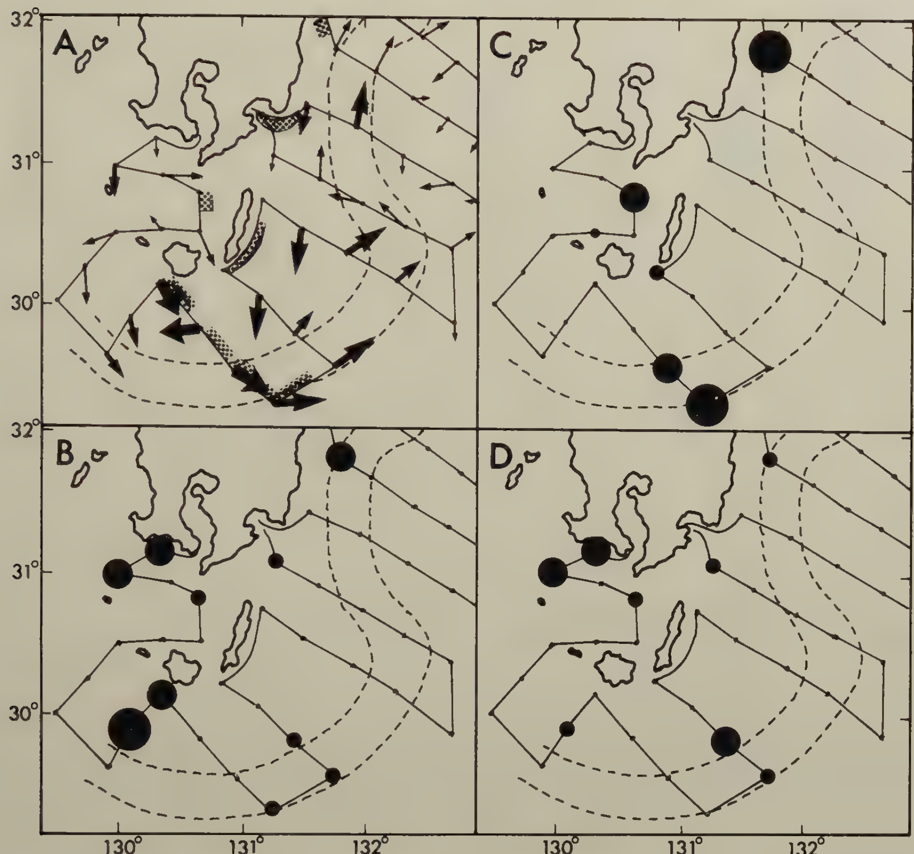


Fig. 6 Distribution of surface water current measured by GEK, shoals of sardine located by echo sounder and eggs and larvae of sardine collected by *Maruchi* net in the Satsunan Area and adjacent waters by R/V SHUNYŌ MARU during Jan. 29 through Feb. 8, 1981.

A: surface water current and echo trace of sardine (modified from Hanaoka, unpublished), B: eggs at early developmental stage, or A-stage defined by Nakai (1962), C: eggs at middle developmental stage, or B-stage, D: eggs at later developmental stage, or C-stage.

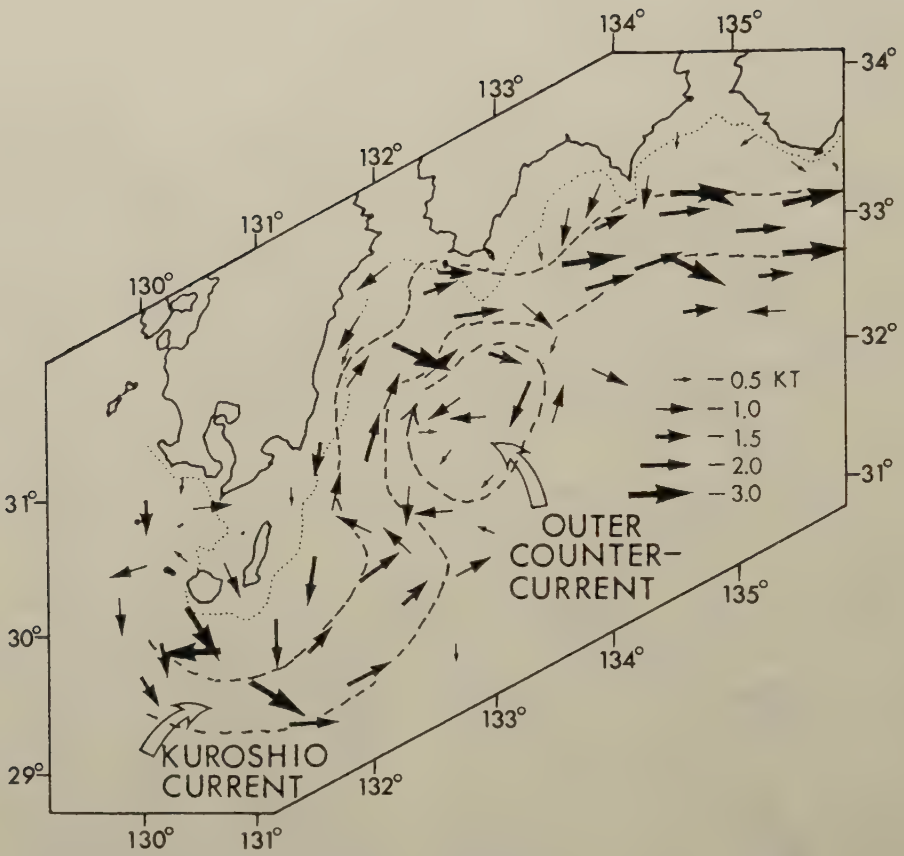


Fig. 7　Distribution of surface water current measured by GEK in the coastal and offshore area from Long. 129° 00′E to Long. 136° 00′E during Jan. 24 through Feb. 8, 1981.

1981年1，2月には黒潮は屋久島南方から種子島東方にかけて離岸した後都井岬で接岸していた。屋久島の北西水域では一定方向の卓越流はみられなかった。種子島の東側の黒潮内側域では反時計回りの環流がみられた（Fig. 6・A）。一方，日向灘東方（四国沖）の黒潮外側域では時計回りの反流がみられた（Fig. 7）。

マイワシ親魚の分布

Fig. 1—6 のAに1976年から1981年にわたる6カ年の1，2月に俊鷹丸の魚群探知機が記録した薩南海域とその周辺における魚群の分布を示した。

魚群の出現位置は後述するマイワシ卵のそれとよく対応する。また，この時期に操業しているまき網漁船，定置網の漁獲物は主としてマイワシ親魚である。したがって，魚群探知機によって記録された魚群の大部分がマイワシ親魚であったと推論できる。この推論に基づきマイワシ親魚の分布を年別に記述する。

1976年1，2月にはマイワシ親魚は薩摩半島の西側，鹿児島湾口，志布志湾沖の沿岸にみられた

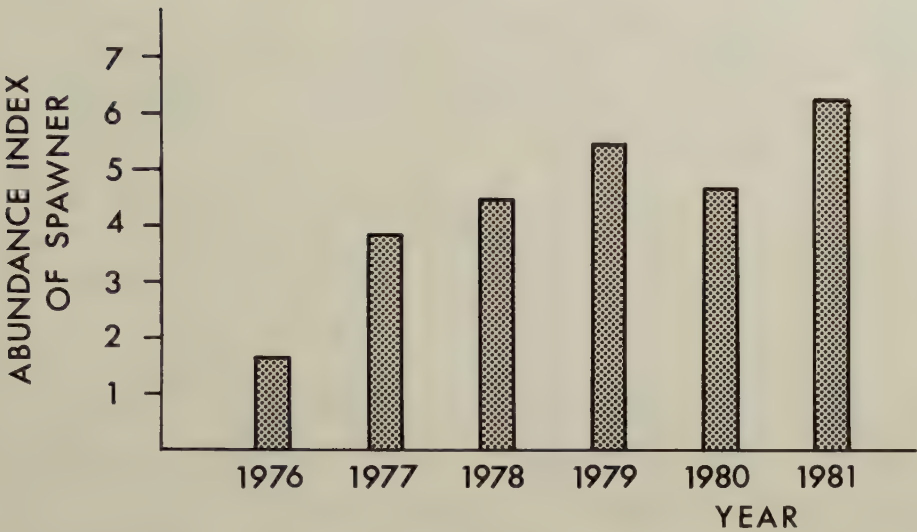


Fig. 8 Abundance index of spawning sardines in the Satsunan Area in January and February, 1976–1981.
Abundance index is given in terms of distance of occurrence on the echo sounder.

(Fig. 1・A)。

1977年1，2月にはマイワシ親魚群は薩摩半島の南側の沿岸で最も多く，他に大隅海峡，種子島の南東でもみられた。種子島南東の親魚群は黒潮とその内側に位置していた（Fig. 2・A)。親魚群の分布域は前年より拡大し，魚群の累積距離で示した存在量指数もやはり増加している（Fig. 8)。

1978年1，2月には親魚群は屋久島，種子島と薩摩半島，大隅半島の中間水域で最も多く，他に鹿児島湾口，志布志湾沖，屋久島の南側のいずれも黒潮内側域でみられた。前年の親魚の分布量と比べて大隈海峡における増加が顕著であった（Fig. 3・A)。

1979年1，2月には親魚群は屋久島と種子島の中間，この南側および種子島東方の黒潮内側域，鹿児島湾口から大隅海峡にかけての水域でそれぞれ多かった。屋久島，種子島の周辺における親魚量が増加した（Fig. 4・A)。

1980年1，2月には親魚群は屋久島，種子島の中間域，種子島の北東と南の黒潮とその内側近傍で最も多く，他に薩摩半島の南岸でもみられた（Fig. 5・A)。

1981年1，2月には親魚群は屋久島，種子島の南方の黒潮とその内側域で最も多く，他に種子島北西，志布志湾口でもみられた。屋久島，種子島南方の親魚群は屋久島の南側の地先からかなり沖合まで延びており，その先端は黒潮に達していた（Fig. 6・A)。

マイワシ卵・仔魚の分布

Fig. 1—5 の B—F と Fig. 6の B—D に 1976年から1981年の 6ヵ年の 薩南海域とその周辺におけるマイワシ卵・仔魚の分布を示した。また，Fig. 9は1981年の薩南海域を含む南西外海の全域における仔魚の分布を示した。

1976年1，2月には初期卵，中期卵，後期卵のいずれも薩摩半島の南側および鹿児島湾と志布志湾の口外で多かった（Fig. 1・B—D）。全長3－6㎜の小型の仔魚は薩摩半島の西方，志布志湾の口外，屋久島の西側で多かった（Fig. 1・E）。全長6—20㎜の大型の仔魚の分布域は小型魚のそれより拡大していた（Fig. 1・F）。

1977年1，2月には初期卵は屋久島北西（Fig. 2・B），中期卵は鹿児島湾口，種子島東方の黒潮本流とその周辺（Fig. 2・C），後期卵は種子島東方の黒潮本流とその内側で多かった（Fig. 2・D）。小型仔魚は種子島東方の黒潮とその周辺（Fig. 2・E），大型仔魚は種子島北東の黒潮内側域，鹿児島湾の口外で多かった。（Fig. 2・F）。

1978年1，2月には初期卵，中期卵，後期卵のいずれも種子島東方から北東の黒潮とその内側に多く，他に屋久島の北西と南東の黒潮内側域でみられた（Fig. 3・B—D）。小型仔魚の分布は卵のそれと類似しているが薩摩半島の西側でやや多かった（Fig. 3・E）。大型仔魚は薩摩半島の西側，志布志湾沖，屋久島南方の黒潮内側域でみられた（Fig. 3・F）。

1979年1，2月には初期卵は種子島南方，日向灘（Fig. 4・B），中期卵は種子島東方に多く他に薩摩半島の西側，屋久島周辺で多かった（Fig. 4・C）。後期卵は都井岬東方の黒潮本流に多く，日向灘南部でもみられた（Fig. 4・D）。薩南海域の卵の分布量は前年よりかなり少なかった。小型仔魚は種子島南東の黒潮外側域と日向灘南部で多く，さらに黒潮本流，薩摩半島の西側，屋久島の北側を含む広い範囲で採集された（Fig. 4・E）。大型仔魚の分布は小型仔魚のそれと類似するが，種子島東方の黒潮とその外側でみられなかったことと採集数が全般的に少なかった点で異なる（Fig. 4・F）。

1980年1，2月には初期卵，中期卵は屋久島から都井岬に至る黒潮周辺で多かった（Fig. 5・B, C）。後期卵と小型仔魚の分布は初期卵，中期卵のそれらとほぼ類似するが，黒潮の外側域ではより沖合でみられた点で異なる（Fig. 5・D, E）。大型仔魚は屋久島北西，種子島東方の黒潮の周辺でみられたが，それらの量はかなり少なかった（Fig. 5・F）。

1981年1，2月には初期卵は薩摩半島の南側，屋久島南方，種子島南東の黒潮とその内側（Fig. 6・B），中期卵は種子島の西と南側の黒潮内側域および南方の黒潮（Fig. 6・C），後期卵は初期卵とほぼ同一水域で多かった（Fig. 6・D）。仔魚は種子島の南東から北東の黒潮とその内側域で多く，それらの分布は日向灘を経て土佐沖に達していた。このことは㋨ネット，㊥ネット，ジェットネットで共通していた。しかし，ジェットネットの結果では他の2種のネットのそれらと異なって，日向灘東方の黒潮外側域でも多くの仔魚がみられた（Fig. 9）。

考　　　察

卵・仔魚の分布は流れおよび親魚の分布によって規定される。この海域における支配的な海流である黒潮本流の位置は，この6ヵ年の間にも大きく変化し，それに伴って沿岸流も年々変動している。

薩南海域の西部では屋久島の北西で黒潮の先端が北偏した1976，1978年にその先端の東側で南下

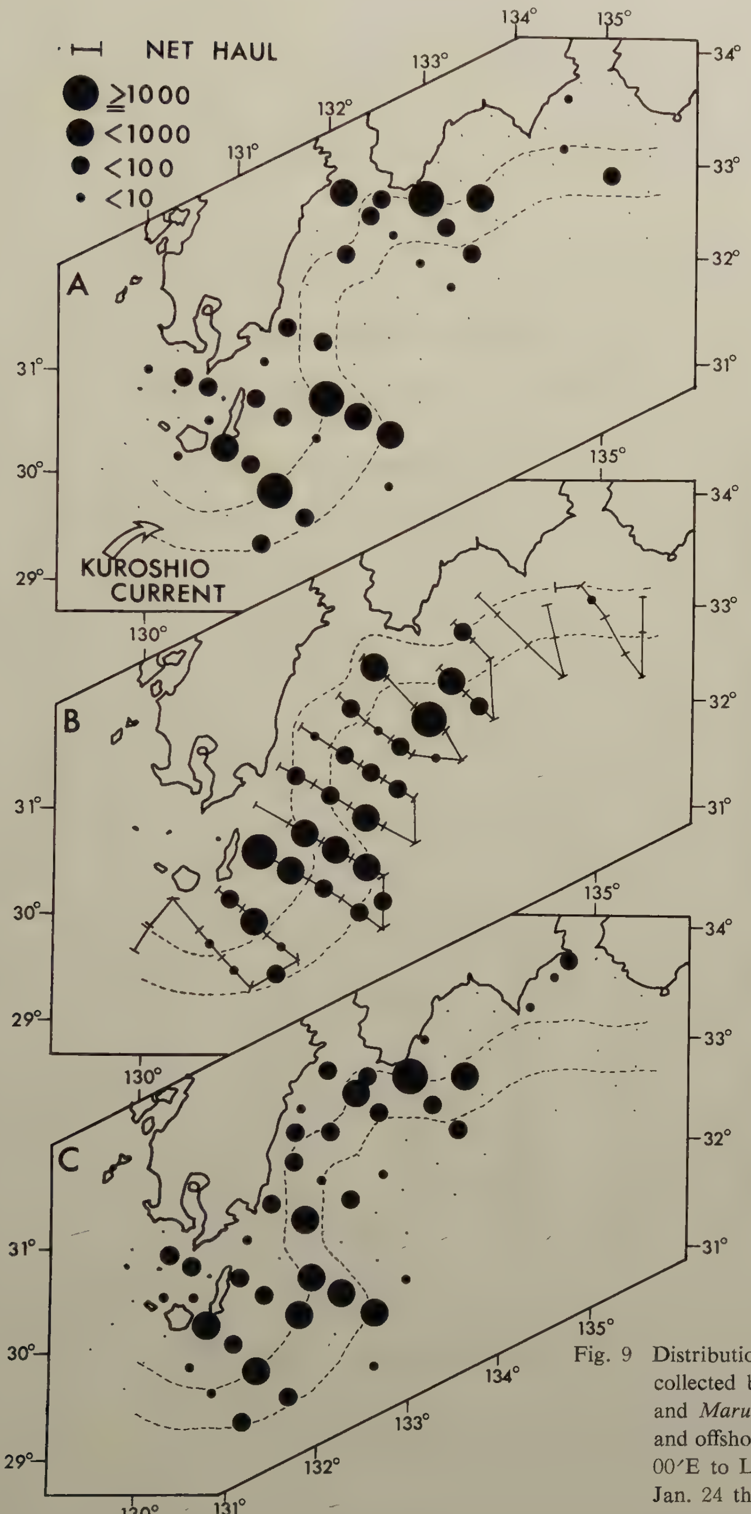


Fig. 9 Distribution of larvae of sardine collected by *Maruchi* net, Jet net and *Marunaka* net in the coastal and offshore area from Long. 129° 00′E to Long. 136° 00′E during Jan. 24 through Feb. 8, 1981.
A : larvae per haul of *Maruchi* net, B : larvae per 1000m³ of Jet net, C : larvae per 100m³ of *Marunaka* net.

流の卓越が目立つ（Fig. 1・A, 3・A)。安井他（1955）によると，大隅海峡を抜ける分枝流は黒潮分枝流と九州西岸からの南下水によって構成されている。1978年の資料はこのことを裏づけている。

屋久島西方で黒潮が南偏した1977，1979—1981年には屋久島北西で弱勢な南成分をもつ流れがみられ，とくに，1979年には南東流が顕著であった（Fig. 2, 4, 5, 6 の A)。この流れは九州西岸からの南下水である。

薩南海域東部では黒潮が種子島と都井岬の東方で離岸した1978，1979年には種子島東方の黒潮内側域で反時計回りの環流，種子島北東の水域で，日向灘からの南下水がみられた（Fig. 3, 4 の A)。黒潮が種子島東方で離岸し，都井岬で接岸した1981年には反時計回りの環流がみられ（Fig. 6・A)，逆に，種子島東方で近づき都井岬で離岸した1977年には日向灘からの南下流が卓越した（Fig. 2・A)。

大隅分枝流は種子島の北側を抜けた後，1978年には東方の黒潮本流に達したが（Fig. 3・A)，1979年には南東に向きを変えて種子島の東側の黒潮内側域にみられる反時計回りの環流に達した（Fig. 4・A)。

黒潮外側域における調査は1980，1981年に充実している。1980年には種子島東方の黒潮外側域では時計回りの反流がみられた（Fig. 5・A)。この反流は1981年にもみられたが，その規模は大きく，種子島東方から日向灘東方あるいは四国沖まで達した（Fig. 7)。

1976年から1981年の6ヵ年の薩南海域におけるマイワシ親魚群は，薩摩半島から大隅半島に至る鹿児島県沿岸域，大隅海峡域，屋久島，種子島周辺域を主分布域としている。薩南海域でマイワシの大規模な産卵が始まった1976年には親魚群は鹿児島県沿岸域に限っていた（Fig. 1・A)。1977年には主分布域は依然薩摩半島南西の沿岸にあったが，分布範囲は大隅海峡および種子島南東の黒潮とその内側域に広がった（Fig. 2・A)。主分布域は1978年には大隅海峡に移り（Fig. 3・A)，1979年には大隅海峡のみでなく屋久島，種子島周辺域に広がった（Fig. 4・A)。親魚群の主分布域と範囲が経年的に南偏する傾向は続く2ヵ年にもみられ，とくに，1981年には親魚群は屋久島，種子島の南方まで分布した（Fig. 5・A, 6・A)。

魚群探知機の記録によるとマイワシ親魚の分布量は1976年に少なくて，1977年以降増加したと思われる（Fig. 8)。鹿児島県枕崎，阿久根の2漁港で1，2月に水揚げされたマイワシは主として親魚である。この漁獲量は1975年に比べて1976年に急増し，その後も高水準にある。とくに，1980，1981年にはそれ以前より多い（Fig. 10)。マイワシの漁獲量は太平洋側のみでなく東シナ海においても1975年以降増加している（Table 1)。ここで取り上げた枕崎，阿久根両漁港の漁船は主に甑島から熊本県牛深までの西薩海域で操業しており，薩南海域で分布する親魚は西薩海域から南下したと考えられている（川上，私信*)。したがって，西薩海域から薩南海域へ南下する親魚量は1976年以降経年的に増加していると考えられる。

1976年から1981年の6ヵ年の薩南海域とその周辺におけるマイワシ卵・仔魚の分布を，流況および親魚群の分布と対応させてみる。

1976年には（Fig. 1)，親魚群は薩摩半島の西側，鹿児島湾口，志布志湾沖の沿岸にみられたが，

* 川上市正，鹿児島県水産試験場

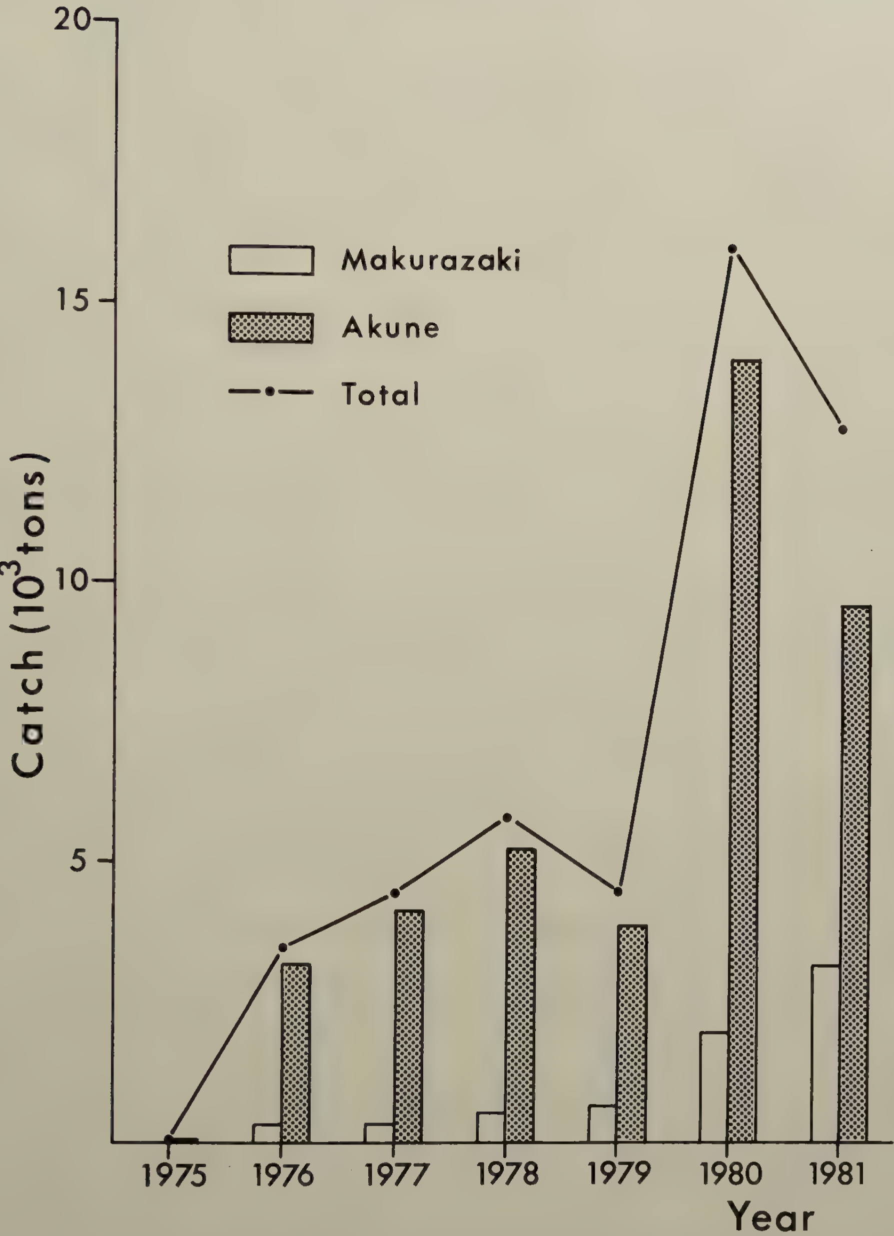


Fig. 10 Amount of catch of spawning sardines landed on the Makurazaki and Akune Fish Markets, Kagoshima Prefecture, in January and February, 1975–1981.

Data from the Kagoshima Prefectural Fisheries Experimental Station (unpublished).

Table 1 Amount of catch of sardine in eight regions around Japan, 1970—1980.
Figure in parentheses denotes percentage to the year's total. Date from Statistics and Information Division of the Ministry of Agriculture, Forestry and Fisheries and its predecessor (1972—1982).

(1000 tons)

Year	Hokkaido Region	North Pacific Region	Middle Pacific Region	South Pacific Region	North Japan Sea Region	West Japan Sea Region	East China Sea Region	Seto Inland Sea Region	Total
1970	0 (0)	2 (12)	10 (59)	2 (12)	0 (0)	2 (12)	1 (6)	0 (0)	17 (110)
1971	0 (0)	2 (4)	43 (75)	6 (11)	0 (0)	4 (7)	0 (0)	2 (4)	57 (101)
1972	1 (2)	3 (5)	26 (46)	12 (21)	1 (2)	11 (19)	1 (2)	2 (4)	57 (101)
1973	1 (0)	126 (43)	103 (35)	15 (5)	4 (1)	40 (14)	2 (1)	5 (2)	296 (101)
1974	11 (3)	152 (43)	84 (24)	8 (2)	5 (1)	76 (22)	6 (2)	11 (3)	353 (100)
1975	11 (2)	241 (46)	147 (28)	9 (2)	7 (1)	63 (12)	26 (5)	21 (4)	525 (100)
1976	63 (6)	393 (37)	222 (21)	65 (6)	34 (3)	170 (16)	106 (10)	15 (1)	1068 (100)
1977	98 (7)	493 (35)	345 (24)	33 (2)	39 (3)	251 (18)	138 (10)	23 (2)	1420 (101)
1978	81 (5)	562 (34)	334 (20)	94 (6)	62 (4)	197 (12)	228 (14)	81 (5)	1639 (100)
1979	109 (6)	568 (31)	258 (14)	81 (4)	93 (5)	332 (18)	302 (17)	75 (4)	1818 (99)
1980	129 (6)	802 (37)	356 (16)	89 (4)	96 (4)	357 (16)	297 (14)	70 (3)	2196 (100)

これらとほぼ同一水域で卵と全長3－6㎜の小型仔魚もみられた。全長6－20㎜の大型仔魚はこれらの水域の他に薩摩半島西方でも多かった。薩摩半島西方の仔魚は九州西岸からの南下水によって運ばれてきたと考えられる。

1977年には（**Fig.** 2），初期卵は親魚群と同様に薩摩半島南側の沿岸で最も多かった。また，種子島東方の黒潮とその内側域の初期卵は，同島南部の東方の黒潮とその内側域で産出されたと思われる。中期卵，後期卵は初期卵と異なって種子島の東方で多かった。これらは鹿児島湾口と大隅海峡および種子島南部の東方で産出され，大隅海峡の南東流と日向灘からの南下水および黒潮によって運ばれてきたと考えられる。小型，大型仔魚も薩南海域の東部で多かった。

1978年には（**Fig.** 3），初期卵，中期卵，後期卵はともに種子島の東方から北東の黒潮とその内側域で多かった。これらの多くは親魚群の分布の中心である大隅海峡域で産出され東流によって運ばれてきたのであろう。この他には志布志湾沖の親魚群に由来し，日向灘からの南下水によって運ばれてきたと考えられる。こうして卵は種子島の東方から北東で収れんしたとみられる。小型仔魚は卵が分布する水域で多いが，他に薩摩半島の西側でも多かった。後者の仔魚は九州西岸から運ばれてきたと考えられる。

1979年には（**Fig.** 4），初期卵，中期卵，後期卵は親魚群の多い鹿児島湾口から大隅海峡にかけての水域と屋久島，種子島の周辺で多かったが，卵の分布量は親魚のそれと比べてかなり少なかった。このことは調査前に大きな産卵がみられなかったためと思われる。小型仔魚は種子島東方の黒潮の周辺で多かった。

1980年には（**Fig.** 5），初期卵，中期卵，後期卵と小型仔魚は屋久島南方から種子島北東の黒潮の周辺で多く，これらはこの水域の黒潮とその内側で産出されたと思われる。ここで産卵された卵・仔魚の多くは黒潮に向う流れによって黒潮本流に入り，それらの一部は黒潮反流に乗って黒潮外側域へ輸送されたと考えてよい。これが発生段階が進むにつれて黒潮外側域に拡散した過程である。

1981年には（**Fig.** 6, 7, 9），初期卵，中期卵，後期卵は薩摩半島の南側，大隅海峡，屋久島の南西から種子島南東の黒潮とその内側域で多かった。これらの分布は，九州西岸からの南下水によって運ばれてきたと思われる薩摩半島の南側の卵を除いて，ほぼ親魚群のそれと一致していた。仔魚は屋久島，種子島の南東から日向灘を経て土佐沖に至る主として黒潮とその内側域で多かったが，ジェットネットの結果では日向灘東方の黒潮反流域でもかなり分布していた。このことは屋久島，種子島の南方の黒潮とその内側域で産出された卵・仔魚の多くは，黒潮によって北東に移動し，その過程で一部のものが黒潮反流に入り込んでいたことを示唆している。

マイワシ卵・仔魚が屋久島，種子島の南東から東方さらに日向灘東方の黒潮外側域に分布する例は，この水域で調査密度が高い1980，1981年の2ヵ年の結果で顕著であった。また，断片的であるが，1977，1979年の2ヵ年でも仔魚が黒潮外側域で分布していた。これらの卵・仔魚のほとんどは屋久島，種子島の南東から北東の黒潮とその内側近傍で産出されたと思われる。

Nakai（1962）は，1930年代のマイワシの豊漁期に薩南海域で産卵されたマイワシ仔魚を太平洋側の黒潮外側域で採集し，薩南海域から輸送されてきた仔魚が，潮岬沖の冷水塊の発生に伴う黒潮の大蛇行によって生じた四国沖の黒潮反流域に収れんしたと報告している。近年では，潮岬から遠

州灘に出現する冷水塊は1975年8月から1980年8月までの5ヵ年にわたってみられた（南西水研海洋部 1980)。したがって，今回調査した1976年から1981年の6ヵ年のうち前5ヵ年は冷水塊の存在期間中にあたる。しかし，今回の結果からはマイワシの卵・仔魚が黒潮の外側域に運ばれることは，潮岬沖の冷水塊の有無にかかわらずみられていた。このことは黒潮の大蛇行の有無にかかわらず黒潮外側のところどころに時計回りの渦流（反流）がつねに存在する（力石　1978）ことと一致している。

浅見他(1967)，工藤他(1981)は薩南海域を主産卵場とするブリ *Seriola quinqueradiata* Temminck and Schlegel の稚仔魚が，主として九州東岸および四国沖の黒潮内側の沿岸で分布するが黒潮外側域でもみられると報告している。前者によると，黒潮外側域に分布するブリ稚仔魚は黒潮がトカラ海域から太平洋へ抜ける際沖合へはねる流れによって薩南海域から輸送されたものと推定している。

渡部（1970）によると，伊豆諸島から房総海域において多量に産卵されたマサバ *Scomber japonicus* Houttuyn の卵・稚仔が黒潮強流帯の沖合側へ拡散する量は少ない。友定(1981）は，伊豆大島の後流域に形成される地形性の渦に集積された卵・稚仔は，黒潮強流帯の内側域（低気圧性シアー域）に閉じこめられて輸送されると述べている。これらの報告は黒潮内側域で産卵された卵・仔魚がその外側の沖合に運ばれることに否定的である。

海域による黒潮外側域への卵・仔魚の輸送に関しては，なお海洋学的な検討を必要とする。ただし，薩南海域におけるマイワシの産卵期である1，2月には北西季節風が卓越するのに対して，伊豆，房総海域の産卵期である4，5月は一定方向の季節風がみられないことに注意する必要がある。浅見（1962）は，表層性である卵・仔魚の分布が風の影響を受けることを指摘している。Hattori and Katoh (1966）が薩南海域で行なった調査では，黒潮内側の沿岸で流した海流瓶の一部が黒潮外側域へ運ばれ反流に乗って沖縄本島，奄見大島に漂着している。これらのことから黒潮内側域で産出された卵・仔魚が，黒潮外側域へ輸送される可能性は考えられる。

要　　約

1976年以降薩南海域にマイワシの産卵場が形成されている。この海域におけるマイワシの発生と補給状態を把握するために，1976年以降毎年1，2月に卵・稚仔調査を行なっている。

ここでは，1976年から1981年の6ヵ年の薩南海域におけるマイワシの産卵場の拡大過程を卵・仔魚の分布から記述し，併せて黒潮の沖合外側域における卵・仔魚の分布の実態について述べた。これらのことを要約すると，以下のようになる。

1）1976年から1981年の6ヵ年に薩南海域におけるマイワシの親魚群は，薩摩半島から大隅半島に至る鹿児島県沿岸域，大隅海峡域，屋久島，種子島の周辺域に分布していた。

2）1976年にはマイワシ親魚群の主分布域は薩摩半島から大隅半島に至る沿岸域に限っていたが，その後年々薩南海域の南部に拡大している。そして，1979年以降親魚群は屋久島，種子島の周辺域に集群し，さらに量的にも増加していると思われる。

3）このようなマイワシ親魚群の分布の変化は流況とともにマイワシ卵・仔魚の分布に影響している。すなわち，1976—1978年の3ヵ年には初期卵は薩摩半島から大隅海峡に至る沿岸域に多く，さらに発生の進んだ卵・仔魚は東方に輸送されていた。1979年には卵の分布量は親魚群のそれよりかなり少なかったが，卵・仔魚は東方に輸送されていた。1980，1981年には卵・仔魚は屋久島種子島の南東水域から黒潮によって北東の下流域へ運ばれていた。

4）卵・仔魚が黒潮によって下流に運ばれる過程でそれらの一部が黒潮の沖合外側域へも輸送されていた。とくに，1981年には屋久島，種子島の南東水域で産出された仔魚が日向灘東方にみられる黒潮反流のほぼ全域で分布していた。

文　　献

浅見忠彦，1962：太平洋南区のカタクチイワシ *Engraulis japonica* (Houttuyn) に関する研究，南海水研報告，(16)，1—55.

浅見忠彦・花岡藤雄・松田星二，1967：産卵および発生初期の生態ならびにモジャコの漁獲および減耗に関する研究，農林水産技術会議研究成果，(30)，1—61.

工藤勝宏・森由基彦・小川和敏・能津純治，1981：流れ藻へのブリ仔の蝟集および流れ藻に伴なわないブリ仔の分布，天然ブリ仔資源保護培養のための基礎調査実験昭和55年度報告，81—101.

Nakai, Z., 1962: Studies relevant to mechanisms underlying the fluctuation in the catch of the Japanese sardine, *Sardinops melanosticta* (Temminck and Schlegel). Japan. J. Ichthyol., **9** (1—6), 1—115.

南西海区水産研究所海洋部，1980：南西海区漁場海況概報，(78)，1—21.

農林省経済局統計情報部，1972—1974：漁業養殖生産統計年報

農林省統計情報部，1975—1976：漁業養殖生産統計年報

農林水産省統計情報部，1977—1982：漁業養殖生産統計年報

Tanaka, H., Y. Imamiya, M. Azeta and M. Anraku, 1968: A hydrodynamic study of a modified model of the Clarke Jet Net. Mar. Biol., **1** (3), 204—209.

Tanaka, H., H. Kasai, Y. Imamiya, S. Kimura, M. Azeta and M. Anraku, 1969: Hydrodynamic and towing characteristics of a modified model of the Clarke Jet Net. Mar. Biol., **2** (4), 297—306.

力石国男，1978：平均的な黒潮の流れ，号外　海洋科学，1 (2)，21—28.

友定彰，1981：マサバ卵・稚仔の輸送に果す黒潮の役割，海洋と生物，(14)，170—175.

内田恵太郎・今井貞彦・水戸敏・藤田矢郎・上野雅正・庄島洋一・千田哲資・田福正治・道津喜衛，1958：日本産魚類の稚魚期の研究　第1集，九州大学農学部水産学第2教室，89p.

渡部泰輔，1970：マサバの発育初期における形態・生態ならびに資源変動に関する研究，東海水研報告，(62)，1—283.

渡部泰輔，1981：マイワシの初期減耗について，漁業資源研究会議報，(22)，67—88. (Watanabe, T., 1981: Mortality of Japanese sardine at early stages of life. Report of Fisheries Resources Ingestigation by the Scientists of the Fisheries Agency, Japanese Goverment, (22), 67—88. Japanese with English abstract).

安井善一・赤松英雄・深瀬茂，1955：昭和30年冬季東支那海海洋観測報告，中央気象台海洋報告，**4** (2)，25—35.

横田滝雄，1953：日向灘・豊後水道のイワシ類の研究，南海区水研報告，(2)，1—251.

別　途　発　表　業　績

Titles of separatedly issued contributions

飼育水の酸素分圧低下によるマダイの血液性状変化

石　岡　宏　子

Changes of haematological constituents of red sea breams exposed to the ambient oxygen reduction by Hiroko ISHIOKA

1981年７月11日受理　業績第111号
日本水産学会誌　48(2)，165～170，（1982）に掲載

マダイは飼育水の酸素量を30分間に，3.75ml/l，4時間に　3.5ml/l　程度急速に減少させると顕著な血液性状変化を示した。最も典型的な反応は血糖値の上昇と血清コーチゾール量の増加であった。また，ヘマトクリット値，ヘモグロビン量も増加の傾向を示した。これらの変化は，4時間に酸素量 1.72ml/l，1.29ml/l 程度の減少時にはみられなかった。

ヘマトクリット値，血糖値，血清ナトリウム量の値の組み合わせによって飼育水の酸素分圧低下時のストレス反応相の分類を試みた。

大阪湾におけるスピオ科の多毛類 *Paraprionospio* sp.（Ａ型）個体群の季節変動と成長

玉　井　恭

Seasonal fluctuation and growth of spionid polychaete *Paraprionospio* sp. (type A) population in Osaka Bay, Japan by Kyoichi TAMAI

1981年11月９日受理　業績第112号
日本水産学会誌　48(3)，401～408，（1982）に掲載

抄　　　録

大阪湾におけるスピオ科の多毛類 *Paraprionospio* sp.（Ａ型）の分布，生息密度の季節変動，成長と，底層環境やベントス群集の季節変動との関係から，本種の富栄養化海域指標としての意義を検討した。夏季の貧酸素化に伴うベントスの減少と餌料となる豊富な有機物移入量が，本種の加入期，低酸素耐性，成長速度，競争種などに関する生理的，生態的な諸特性とあいまって，夏から秋にかけての本種の爆発的な増殖を引き起すと考えられる。このことは本種が富栄養化海域の指標として利用できることを示している。

スズキの鰭および鱗の発達（英文）

福 原　　修・伏 見　　徹

Development of fins and squamation in the Percichthyid fish, *Lateolabrax iaponicus*

by Osamu FUKUHARA and Toru FUSHIMI

1982年７月28日受理　業績第123号
魚類学雑誌　29(2), 13～18,（1982）に掲載

抄　　録

スズキの幼期における外部形態，特に鰭の発達と鱗の形成を明らかにするため，人工飼育で得た標本をアリザリンで染色し観察した。鰭については各鰭の軟条の分節，分枝について調べた。各軟条の分節は18㎜までに完了し，各鰭の基本的構造は完成する。そして分枝は体長40㎜までに完了する。

鱗の発生は，体長19㎜頃に体側正中線上の尾柄直前付近から始まり，鱗域を各方向に拡げながら体長35㎜頃から体側全面を覆う個体が現われる。こうした外部形態の変化は，天然標本による観察結果と比較して鰭についてはほぼ同様であったが，鱗については体側全面を覆う時期に差異が認められた。

平太洋中部・南部沖合底びき網漁業漁場別漁獲統計資料、昭和56年１～12月

通 山 正 弘・堀 川 博 史・岸 田 周 三

Taiheiyō chūbu nanbu okiai sokobikiami gyogyō gyojōbetsu gyokaku tōkei shiryō Shōwa 56-nen 1-12 gatsu（Catch statistics by fishing area on offshore trawl fisheries in central and southern Pacific waters along Japan, January-December 1981）by Masahiro TŌRIYAMA, Hiroshi HORIKAWA and Shūzō KISHIDA*

* see footnote of the table of contents on the back cover.

1982年８月12日受理　資料第12号　113 p.

1981年１～12月の間に太平洋中部・南部海域を漁場とする沖合底びき網漁業者が農林水産大臣に提出した漁獲成績報告書の集計結果である。内容は１そうびき，２そうびきという漁業種類ごとの漁場，ひき網回数，主要種の漁獲量などの要約，並びに船籍県，漁業種類，漁船規模別着業統数，航海数，操業日数，ひき網回数，漁獲量などの月別統計，漁業種類ごとの緯経度10分ますめ別，月別ひき網回数，魚種別漁獲量などの月別統計，及び資源量指数，有効漁獲努力量，漁獲努力の有効度などの一覧表という３種類の統計表である。

1そうびき漁船は過去10年間ほとんど大型化していない。地域的には愛知，三重，和歌山県船が40トン級，徳島，高知県船が30トン級である。それぞれ漁具，地形によって船の大型化が制限されていると考えられる。2そうびき漁船は徳島県では10年前と同じく28トン級であるが，高知県から鹿児島県に至る地域では大型化が進み60〜80トン級となった。

もっとも良く利用する漁場は1そうびき漁船では熊野灘，遠州灘，土佐沖，紀伊水道等，2そうびき漁船では豊後水道沖，北日向灘等である。漁獲量は1そうびき漁船4,629トン，2そうびき漁船13,899トンでそれぞれ前年比9％，6％の増加であった。

〔非売品〕

昭和58年３月27日　印　刷　　昭和58年３月30日　発　行

広島県佐伯郡大野町丸石7782－9

発行者　南西海区水産研究所

桑谷幸正

印刷所　株式会社　中本本店印刷

南西水研報
Bull. Nansei Reg. Fish.
Res. Lab. No 15, 1983

Contents

* In Japanese without English title. The Japanese title is given in italics with its English translation in parentheses.